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Synthesis of Selenium- and Tellurium-Containing Tryptophan Analogs for the Elucidation of Protein Structure and Function

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4-(6H-selenolo[2,3-*b*]pyrrolyl)-L-alanine
[6,7]SeTrp

4-(6H-telluro[2,3-*b*]pyrrolyl)-L-alanine
[6,7]TeTrp

L-tryptophan

- B.S. Biochemistry, TTU, 2001.
- M.S. Chemistry, TTU, 2003.
- GRA, Bioscience Division, LANL, 2002-2004.
- Ph.D., Organic Synthesis, University of Cincinnati, 2009.
- NIH Post-doctoral Fellow, Vanderbilt University, 2009-2011.
- Visiting Scholar, Vanderbilt University, 2011-2015.
- Assistant Professor, Chemistry, Belmont University, 2011-current.
- Guest Scientist, Bioscience Division, LANL, 2014-current.

Other research projects

ROS interactions with protein-tyrosine residues produce *p*-peroxy and *p*-hydroxy tyrosine electrophiles (Michael adducts).

Synthesis of multivalent carbohydrate ligands for the detection and differentiation of pathogens.

Goals

- Rational design, synthesis, and characterization of Se- and Te-containing tryptophan analogs.
 - Synthesis of selenonphenes and telluorophenes
 - Enzymatic coupling of serine side chain using tryptophan synthase
- Bio-incorporate heavy atom tryptophan analogs into selected proteins.
- X-ray and NMR structural studies of selected proteins.
- Rationally design drugs based off active sites and other functionally important protein residues.
- Design novel proteins that have new functional properties.

Methods of solving protein structures

**Percentages based on current Protein Data Bank entries*

Methods continued

- X-ray crystallography
 - Molecular replacement (MR) – homologous protein with >40% sequence identity.
 - Isomorphous replacement (SIR/MIR) – soak crystals in salts of heavy atoms and compare diffraction patterns to native crystal (deduce phases). (often changes cell dimensions)
 - **Multiwavelength anomalous dispersion (MAD)** - SeMet
 - Single anomalous dispersion (SAD) - SeMet
- NMR
- SOLVE/RESOLVE - computational

Overview of the X-ray Crystallographic Method

The “phase problem” of x-ray crystallography

- X-ray diffraction patterns from crystals of proteins contain sufficient information to define atomic structure.
- Atomic positions are inextricable without having electron-density images.
- Diffraction measurements provide amplitudes (probability proportional to the square of the amplitude of this wave), but not phases for the diffracted waves, which is needed for the computation of electron density.
- Phase problem is reduced by determining the position of a few heavy atoms (anomalous scattering or dispersion).
- Different atoms contribute to the scattered intensity in proportion to the square of the number of electrons they contain.

A solution to the “phase problem”

- Develop heavy atom (electron rich) containing amino acids (Se- and Te-containing tryptophan analogs).
- The MAD technique yields anomalous scattering of the electron rich metalloid atoms, thus allowing the phases to be determined from a single crystalline species.
- Selenomethionine (SeMet) is the gold standard.

Use of selenomethionine (SeMet)

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☐ 1. Purification and identification of Se-containing antioxidative peptides from enzymatic hydrolysates of Se-enriched brown rice protein

By: Liu, Kunlun; Zhao, Yan; Chen, Fusheng; et al.
FOOD CHEMISTRY Volume: 187 Pages: 424-430 Published: NOV 15 2015

GO TO ULRICH'S

View Abstract

Times Cited: 0
(from All Databases)

☐ 2. Effects of different levels of dietary selenium nanoparticles on growth performance, muscle composition, blood biochemical profiles and antioxidant status of common carp (Cyprinus carpio)

By: Ashouri, Samyar; Keyvanshokoh, Saeed; Salati, Amir Parviz; et al.
AQUACULTURE Volume: 446 Pages: 25-29 Published: SEP 1 2015

GO TO ULRICH'S

View Abstract

Times Cited: 0
(from All Databases)

☐ 3. Reactivity of selenium-containing compounds with myeloperoxidase-derived

Times Cited: 0

Ideal heavy atom derivative

- Stability is important both chemically and structurally.
 - SeMet and **TeMet** are prone to oxidation (no longer useful).
 - SeTrp and TeTrp should be more stable as the heavy atom is part of an aromatic ring system.
- Low frequency of occurrence in the asymmetric unit.
 - Phase determination generally fails if more than ~4 heavy atoms are present in the repeating unit of the crystal.
 - Methionine is the 4th most rare AA (2.6%).
 - Tryptophan is the most rare AA (1.3%).
 - Applicable to a larger range of proteins.
- Se-77 and Te-125 are NMR active isotopes (large chemical shifts that are highly sensitive to the local structural environment).
- Use SeTrp and TeTrp alongside SeMet (more tools in the toolbox).

Syntheses of selenophene pyrroles

- 1st reported synthesis for selenolo[3,2-*b*]pyrrole and selenolo[2,3-*b*]pyrrole were published by Paulmier and coworkers in 1978.
- More recent attempts to improve the 35% and 38% yields were carried out by Phillips and coworkers in 1999.
- The selenophene pyrroles were constructed through slight modifications of the final step of Paulmier's original method.

Phillips modified Paulmier method for the construction of 4-(6H-selenolo[2,3-*b*]pyrrolyl)-L-alanine

Reagents: (a) N₃CH₂CO₂Et, NaOEt, 0°C, 2h (60%); (b) xylenes, reflux, 10 min, (crude yield 85%);
(c) KOH, heat (crude yield 100%); (d) glycerol, 160-170°C, 45 min (crude yield 75%).

Phillips et al., 1999.

Phillips modified Paulmier method for the construction of 6-(4H-selenolo[3,2-*b*]pyrrolyl)-L-alanine

Reagents: (a) $\text{N}_3\text{CH}_2\text{CO}_2\text{Et}$, NaOEt, 0°C , 2h (55%); (b) xylenes, reflux, 10 min, (crude yield 85%);
(c) KOH, heat (crude yield 100%); (d) glycerol, $160\text{--}170^\circ\text{C}$, 45 min (crude yield 70%).
Phillips et al., 1999.

Old synthesis of [6,7]SeTrp; LANL

Annulation data for various Lewis acids

Acid	Conditions ^a	Yield of 2	Yield of 3
Amberlyst	Reflux 24hr.	72 ^c	5
TiCl ₄	-78 °C	48 ^b	0
TiCl ₂ (O-isopropyl) ₂	-78 °C to RT	58 ^b	0
TiCl ₂ (O-isopropyl) ₂	-78 °C to -10°C	21 ^c	0
TiCl ₂ (butoxide) ₂	-78 °C	0 ^b	0
BF ₃	-78 °C to -55 °C	0 ^b	0
SnCl ₄	-78 °C to RT	0 ^b	0
ZnCl ₂	-20 °C to RT	0 ^b	0

a) No starting material recovery yields were obtained for the above reactions. b) Small scale reaction. c) Performed on 4.75 g.

New synthesis of [6,7]SeTrp; LANL

Table 2.6. ¹H-NMR Ts-selenide data.

Proton	Shift	Peaks	J
Assignment	(ppm)		Coupling (Hz)
a	7.87	d, 2H	8.35
b	7.51	dd, 1H	3.40, 1.79
c	7.30	d, 2H	8.22
d	6.48	dd, 1H	3.35, 1.78
e	6.27	t, 1H	3.38
f	5.09	t, 1H	4.55
g	3.89	m, 4H	3.89
h	2.95	d, 2H	2.95
i	2.42	s, 3H	

```

DE 198.29 used
TE 300.0 K
D1 1.00000000 sec
P1 4.00 usec
SFO1 300.1314913 MHz
NUC1 1H
PR1 0.00 dB

F2 - Processing parameters
SI 65536
SF 300.1299876 MHz
WDW EM
SSB 0
LA 0.10 Hz
GB 0
PC 1.00

```

Current Data Parameters

NAME TsSelpyrrole
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

Date_ 20020729
Time 23.00
INSTRUM spect
PROBHD 5 mm Multino
PULPROG zgpg
TD 8192
SOLVENT CDCl3
NS 249
DS 4
SWH 28571.428 Hz
FIDRES 3.487723 Hz
AQ 0.1434100 sec
RG 65536
DW 17.500 usec
DE 6.00 usec
TE 300.0 K
d11 0.03000000 sec
PL12 18.00 dB
D1 1.00000000 sec
P1 19.00 usec
SFO1 57.2559060 MHz
NUC1 77Se
PL1 0.00 dB
CPDPRG2 waltz16
PCPD2 106.00 usec
SFO2 300.1314913 MHz
NUC2 1H
PL2 18.00 dB

F2 - Processing parameters

SI 65536
SF 57.2392603 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00

169.972

S#: 1849 IT: 1.56 ST: 0.59 #At: 10

SE: 1.23e+007

C₁₅H₁₇NO₄SSe

Exact Mass: 387.00

Mol. Wt.: 386.33

C, 46.63; H, 4.44; N, 3.63; O, 16.57; S, 8.30; Se, 20.44

Proton	Shift	Peaks	J
Assignment	(ppm)		Coupling (Hz)
a	7.87	d, 2H	8.57
b	7.61	dd, 1H	5.79, 0.92
c	7.34	dd, 1H	3.40, 0.91
d	7.29	d, 2H	8.31
e	7.19	d, 1H	5.79
f	6.62	d, 1H	3.39
g	2.40	s, 3H

Current Data Parameters
NAME: TsHeterocycle
EXPNO: 2
PROCNO: 1

F2 - Acquisition Parameters

Date_: 20080108
Time: 21.08
INSTRUM: spect
PROBHD: 5 mm Multino
PULPROG: zgpg
TD: 32768
SOLVENT: CDCl3
NS: 2218
DS: 4
SWH: 45577.912 Hz
FIDRES: 1.453107 Hz
AQ: 0.2564020 sec
RG: 32768
CH: 10.875 usec
DE: 6.00 usec
TE: 300.0 K
d11: 0.03000000 sec
PC12: 18.00 dB
d1: 1.00000000 sec
P1: 15.00 usec
SFO1: 57.2678060 MHz
NUC1: 77Se
P11: 0.00 dB
CPCPRG2: waltz16
PCPD2: 56.00 usec
SFO2: 300.1314913 MHz
NUC2: 1H
P12: 18.00 dB

F2 - Processing parameters

SI: 65536
SF: 57.2342603 MHz
WDW: EM
SSB: 0
LB: 6.00 Hz
GB: 0
PC: 1.00

507.827

Duane #197-227 RT: 2.23-2.51 AV: 31 NL: 6.25E5
T: + p-ESI Full ms [85.00-400.00]

1 in DMSO-d₆

Table 2.10. ¹H-NMR Heterocycle data.

Proton	Shift	Peaks	J
Assignment	(ppm)		Coupling (Hz)
a	7.39	dd, 1H	5.65, 0.99
b	7.20	d, 1H	5.65
c	6.99	dd, 1H	2.95, 0.99
d	6.33	d, 1H	2.95

Current Data Parameters
NAME TsBIScyclo
EXPNO 15
PROCNO 1

F2 - Acquisition Parameters
Date_ 70020807
Time 3.18
INSTRUM spect
PROBHD 5 mm Multicou
PULPROG zgpg
ID 8192
SOLVENT CDCl3
NS 31849
DS 4
SWH 5510.417 Hz
FIDRES 0.796729 Hz
AQ 0.5291956 sec
RG 32768
DN 76.800 usec
DE 6.00 usec
TE 300.0 K
d11 0.03000000 sec
PL12 18.00 dB
D1 1.00000000 sec
P1 19.00 usec
SFO1 57.2649268 MHz
NUC1 77Se
PL1 0.00 dB
CPDPRG2 waltz16
PCPDZ 106.00 usec
SFO2 300.1314913 MHz
NUC2 1H
PL2 18.00 dB

F2 - Processing parameters
SI 65536
SF 57.2392603 MHz
WDW RM
SSE 0
LB 6.00 Hz
GB 0
PC 1.00

Se-77 NMR of the selenolo[2,3-b]pyrrole
8-6-02

MS: 21 120 7.44 271 0.59 881 10

MS: 2.75e+006

Tryptophan Synthase

- $\alpha_2\beta_2$ heterodimer
- $\alpha\beta\beta\alpha$ arrangement
 α – 28,700 Da
 β – 43,000 Da
- Pyridoxal phosphate dependent.
- Catalyzes the last two steps in the biosynthesis of L-tryptophan.
- Accommodates many different indole derivatives.

*picture from the protein data bank website

Enzymatic synthesis of [6,7]SeTrp

- 50 mg (0.2 mmol) selenolo[2,3-*b*]pyrrole (direct or in DMSO).
- 40 mg (0.4 mmol) L-serine (labeled or unlabeled).
- 0.5 mg pyridoxal-5-phosphate (PLP).
- 1 ml (13 mg) *S. typhimurium* tryptophan synthase in 60 ml degassed 0.1 M Tris-HCl buffer pH 7.8 (or 0.1 M K₂HPO₄ buffer, pH 7.8).
- 37°C, dark, gently swirl, and monitor by TLC and ¹³C-NMR.
- Purification (HPLC or reverse-phased cartridge).

Boles et al., 2002

Hatch, 2003

Hatch et al., 2004

Fig. 9 Initial Size Exclusion Column

Fig. 10 Ion Exchange Column

Fig. 11 Final Size Exclusion Column

Fig. 8 SDS Gel for TrpSyn purification

Figure 4.13. $[M+H]^+$ ^{13}C -labeled [6,7]SeTrp.

^{13}C -labeled
[6,7]SeTrp

$\text{C}_8^{13}\text{CH}_{10}\text{N}_2\text{O}_2\text{Se}$

Exact Mass: 258.99

Mol. Wt.: 258.14

C, 42.26; H, 3.90; N, 10.85; O, 12.40; Se, 30.59

[6,7]SeTrp-Dihydrofolate Reductase

- Excellent test vehicle for unnatural amino acids
- 18 kDa
- 5 Trp and 5 Met residues
- 50 mg pure [6,7]SeTrp-DHFR
- 100% bio-incorporation via ICP and AA spectroscopy.
- No loss of activity over prolonged periods.

*picture from the protein data bank website

Bio-incorporation of [6,7]SeTrp into Lignan Peroxidase (LiP)

- Extracellular heme protein.
- Cellulosic ethanol is a type of bio-fuel produced from biomass (lignocellulose).
- Non-food biomass is a viable raw material for bio-fuels.
- Need to remove lignin, which is as much as 25 wt % of non-food biomass.
- Nature uses oxidative enzymes (peroxidases) to breakdown lignin.
- LiP has a catalytic Trp residue (Trp171) that is hydroxylated at the β C position.
- Enhance activity by replacing Trp171 with the more polarizable [6,7]SeTrp?

Tryptophan Auxotroph

- We will create a Tryptophan auxotroph using a synthetic antisense RNA (asRNA) construct that will translationally block the expression of the Tryptophan Synthase (TryB).
- The asRNA will be expressed constitutively from the same expression vector expressing the Lignin Peroxidase (LipP).

Conclusions

- [6,7]SeTrp can be efficiently synthesized by these procedures and appears to be rather stable when protected from aeration and light.
- High-level incorporation of [6,7]SeTrp into recombinant DHFR with no loss of activity.
- [6,7]SeTrp and [6,7]TeTrp have the potential of becoming useful tools in the determination of three-dimensional protein structures.
- Stable isotope labeled [6,7]SeTrp and [6,7]TeTrp can be useful for the determination of structures and dynamics of proteins via NMR spectroscopy.
- Potentially useful for enhancing activity of LiP

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Bioscience Division, Los Alamos National Lab.

Department of Chemistry and Physics, Belmont University.

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