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The Design and Synthesis of a Novel Thiamine Cofactor for Potential Biocatalysis

PhD Doctoral Thesis of

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Preface

The research in this thesis of 260 pages is, to the best of my knowledge, original except where due reference has been made to previous work.

R. A. J. Tinson

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Acknowledgements

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Abbreviations

Ac	Acetyl
Å	Ångstrom
Aq	Aqueous
Ar	Aryl
Abs	Absorption
ADH	*Alcohol dehydrogenase*
AIBN	Azobisisobutyronitrile
Atm	1 atmosphere = 10 Pa
BBN	9-Borabicyclo[3.3.1]nonane
BINOL	1,1-bi-2,2-napthol
BOC	*tert*-butyloxy carbonyl
B.p	boiling point
Bn	Benzyl
BSA	Bovine serum albumin
Bu	Butyl
Bz	Benzoyl
Cat.	Catalytic
CCE	Corey-Chaykovsky epoxidation
Conc/cc/c.	Concentration
CM	Cross Metathesis
CuAAC	Copper catalysed Azide-Alkyne Cycloaddition
d	Days
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1, 8-Diazobicycloundec-7-ene
DCM	Dichloromethane
DIBAL-H	Diisobutylaluminium hydride
DIP-Cl	Diisocamphenyl borane chloride
DIPEA	Diisopropylethylamine
Dil.	Dilution

DMAP	Dimethylaminopyridine
DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPPA	Diphenylphosphoryl azide
E2	Bimolecular Elimination
Enz	Enzyme
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol
Eq.	Equivalent
EWG	Electron withdrawing group
FGI	Functional group interconversion
FMO	Frontier molecular orbital
G2	Grubbs 2nd Generation catalyst
h	hours (reaction time)
HETDP	2-(Hydroxyethyl) thiamine diphosphate
Hgmm	millimetre of mercury (760Hgmm = 1atm = 760 Torr)
HOMO	Highest occupied molecular orbital
HPLC	High-pressure liquid chromatography
hν	Irradiation with light
HWE	Horner-Wadsworth-Emmons
IPA	Isopropylalcohol
IR	Infra-red spectroscopy
L	Litre
L*	Ligand
LA	Lewis acid
LDA	Lithium diisopropylamide
LBD	Lipoyl binding domains
L/H/S	Left hand side

LUMO	Lowest unoccupied molecular orbital
LTDP	2-(2-Lactyl)thiamine diphosphate
M	Molar
MBH	Morita-Baylis-Hillman
m-CPBA	*meta*-Chloroperbenzoic acid
MES.KOH	2-(*N*-morpholino) ethanesulfonic acid
Me	Methyl
MeOH	Methanol
Mins	Minutes
mL	milliLitre
mM	MilliMolar
Mmol	millimoles
Ms	Mesityl
MS	Mass spectrometry
MVK	Methyl vinvyl ketone
NADH	Nicotinamide adenine dinucleotide
PDC	*Pyruvate decarboxylase*
PDH	*Pyruvate dehydrogenase*
Pr	Propyl
Psi	Pound per square inch
Py	Pyridine
r.t.	Room temperature
secs	Seconds
SM	Starting material
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutyl ammonium iodide
TBAPP	Tetrabutylammonium pyrophosphate
TBDMS	Tetrabutyldimethylsilane
ThDP	Thiamine diphosphate
THF	Tetrahydrofuran

TMEDA	Tetramethylethyldiamine
TMG	1,1,3,3-Tetramethylguanidine
TPP	Thiamine pyrophosphate
Ts	Tosyl
Uv/Vis	Ultraviolet/Visible
µM	MicroMolar
µL	MicroLitre
^{n}Bu	n-butyl
Δ	Heat
e.e.	Enantiomeric excess
m	Meta
o	Ortho
p	Para

Abstract

The design and application of enzyme mimics has received more attention due to requirement of green chemistry applications within industry and academia. One way of introducing these modifications is via a cofactor, due to their location within an enzyme's active site. A number of crucial biochemical reactions in the cell require not only the enzymes for catalysis, but also the organic cofactors or metal ions. It is therefore advantageous to utilize this common structural relationship to model a novel enzyme – cofactor system capable of undergoing chemistry not commonly undertaken by this enzyme, and which could be beneficial to the synthetic chemist. Recent research has concentrated on the synthesis of unnatural vitamin B_1 motifs containing a central 1,4 - triazole motif (figure **1**) first synthesised by Leeper in 2006.

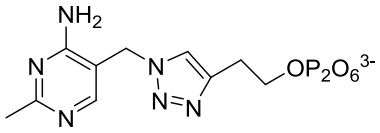

Figure 1: 1,4 – disubstituted triazole ThDP

Synthesis of a cofactor that could theoretically carry out alternative reaction pathways and invoke novel enzyme-substrate pathways, whilst regenerating the cofactor *in situ* was envisaged (figure **2**). Structural analogues of this coenzyme could thus be synthesised to tailor different products, thereby promoting high yields, high stereo/regiochemical control and reduced costs for industrial application.

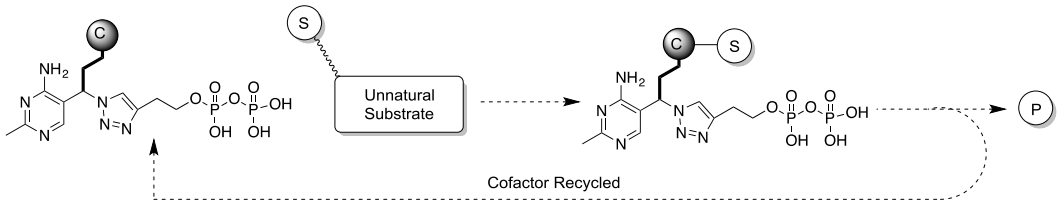

Figure 2: Proposed novel bio catalytic pathway based on 1,4 -ThDP scaffold

The multi-step synthesis of some model compounds and their testing in *pyruvate decarboxylase* enzyme was successfully completed. Results indicated that only smaller chains are accommodated in the active site and initial attempts to synthesis a tertiary amine tether proved difficult because of intramolecular cyclisations. However, a potential novel route to benzoxyazepines was uncovered by an acid catalysed deprotection, cyclisation, elimination step. Enantiopure synthesis was also carried out, and an initial high ee% was observed, but conditions for this require further development. Lastly, the synthesis of a new difluorophosphate isostere for the diphosphate group was developed in good yield for a potential 1,4-CuAAC of our compound for further biological evaluation in *PDC* enzymes.