HIV-1 Integrase Inhibitors: A Formal Total Synthesis of Lithospermic Acid and Synthetic Studies Towards Integramycin

A Thesis submitted in fulfilment of the

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Doctor of Philosophy

By

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Hofstadter's Law: It always takes longer than you expect, even when you take into account Hofstadter's Law.

– Douglas Hofstadter

DECLARATION

This thesis is a summary of work carried out in the School of Chemistry, The University of Sydney, and the Ian Wark Laboratories, CSIRO Clayton, under the supervision of Dr Mark J. Coster, A/Prof Katrina A. Jolliffe and Dr Paul Savage between March 2004 and November 2007.

This thesis contains no material previously published or extracted in whole or in part from a thesis presented by me for any other degree or diploma. No other person's work has been used without due acknowledgement and every effort has been made to acknowledge previously published material. This thesis contains less than 80000 words.

Sections of original work described in this manuscript have been published in a peer-reviewed scientific journal, namely:

"Regioselective Reduction of 3-methoxymaleimides: An Efficient Method for the Synthesis of Methyl 5-hydroxytetramates" Issa, F; Fischer, J; Turner, P; Coster, M.J.; *J. Org. Chem.*, **2006**, *71*, 4703–4705.

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ABSTRACT

This thesis describes synthetic studies towards the HIV-1 integrase inhibitory natural products lithospermic acid and integramycin, resulting in a formal total synthesis of the former.

A modular, flexible and convergent synthetic strategy to lithospermic acid was devised. In this approach, a Sonogashira coupling was used to unite the C_1–C_7 and C_{20}–C_{27} fragments that were subsequently manipulated to then participate in the key step of the synthesis, a palladium-mediated carbonylative annulation. Reduction of the benzofuran nucleus with magnesium in methanol then provided the desired dihydrobenzofuran core of lithospermic acid. Various protecting group strategies were investigated to complete this sequence in an efficient manner. Further synthetic manipulations afforded the complete C_1–C_9/C_{19}–C_{27} fragment, which was united with the C_{10}–C_{18} fragment to deliver the entire carbon skeleton of lithospermic acid. A two step deprotection sequence was undertaken, however, complications with the final deprotective step prevented definitive proof that the total synthesis of lithospermic acid had been achieved. An alternate protecting group strategy was sought, and a formal total synthesis of lithospermic acid was achieved by intercepting an advanced intermediate from a previous total synthesis. Several strategies for the enantioselective synthesis of the dihydrobenzofuran core of lithospermic acid were evaluated, however, none proved successful.

A synthetic route towards the tetramic acid subunit of integramycin was also investigated. 3-Methoxymaleimide was constructed using known chemistry, and the regioselective reduction of this ring system was developed. Attempts to further functionalise this ring system were thwarted by difficulties associated with handling. The scope of the regioselective reduction was investigated on an array of *N*-substituted methoxymaleimides with the procedure found to be generally high yielding and highly regioselective.

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Experimental

ABBREVIATIONS

%	percentage yield
Δ	reflux
°C	degree/s Celsius
Å	Angstrom, 10^{-10} metres
Ac	acetyl
AIDS	acquired immunodeficiency syndrome
Anal.	analysis
aq.	aqueous
Ar	aryl or argon
Asp	aspartic acid
BAr_F	$B(2,3\text{-}(CF_3)_2C_6H_3)_4$
BBN	9-Borabicyclo[3.3.1]nonane
Bn	benzyl
br	broad
Bu	butyl
C	cysteine
CCR5	chemokine receptor 5
CC_{100}	cytotoxic concentration for 100% of cells
ca.	circa (approximately)
calc.	calculated
cm^{-1}	reciprocal centimeters
coe	cyclooctene
Cys	cysteine
δ	chemical shift
D	aspartic acid
d	doublet/s
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DKA	diketoacid
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide

DNA	deoxyribonucleic acid
dppp	1,3-bisdiphenylphosphinopropane
DIBAL–H	diisobutylaluminium hydride
E	glutamic acid
ee	enantiomeric excess
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EI	electron impact
equiv.	molar equivalent(s)
Et	ethyl
eV	electron Volts
Glu	glutamic acid
g	gram
H	histidine
h	hour/s
HAART	Highly active anti-retroviral therapy
His	histidine
HIV	human immunodeficiency virus
HRMS	high resolution mass spectrometry
Hz	Hertz
IC_{50}	concentration required to inhibit a given biological process by 50%
i-Pr	isopropyl
IN	integrase
IR	infrared
J	coupling constant
K	Kelvin
L	litre
μ	micro
m	multiplet
M	molar
M^+, $M^{\bullet+}$	molecular ion
Me	methyl
min	minutes
MHz	megahertz
mmol	millimole
mol	mole

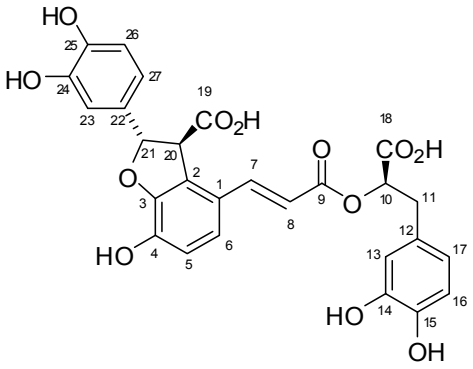
m.p.	melting point
MS	mass spectrometry
m/z	mass to charge ratio
n-BuLi	*n*-butyllithium
NMR	nuclear magnetic resonance
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
Ph	phenyl
PI	protease inhibitor
PIC	pre-integration complex
PMHS	polymethylhydrosiloxane
ppm	parts per million
p-TsOH	*para*-toluenesulfonic acid
pyr	pyridine
q	quartet
quant.	quantitative (yield)
R	unspecified functional group
R_f	retention factor
RNA	ribonucleic acid
rt	room temperature (25 °C)
s	singlet
t	triplet
t-AmOH	*tert*-amyl alcohol
TBAF	tetrabutylammonium fluoride
TBDPSE	*tert*-butyldiphenylsilylethyl
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
tlc	thin layer chromatography
TMS	trimethylsilyl; tetramethylsilane
Ts	tosyl
USFDA	United States Food and Drug Administration
v/v, w/v	volume per volume, weight per volume

COMPOUND NUMBERING

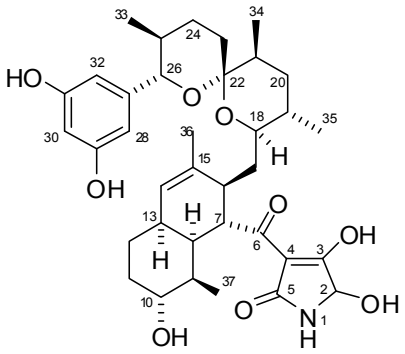
In general, the naming and numbering priorities used throughout this manuscript are those set down by IUPAC. Otherwise, the numbering system of lithospermic acid (**21**) and integramycin (**47**), set forth by Kelley *et al.*[43] and Singh *et al.*[54] respectively, are employed and are shown below.

When discussing the 2,3-disubstituted-2,3-dihydrobenzofuran ring systems, the designations *cis*- and *trans*- refer to the relative configuration of the substituents, and unless otherwise noted, refer to the racemate. Further to this, diastereomeric compounds are assigned the same compound number but are distinguished by the stereochemical descriptor in the prefix.

When discussing the tetramic acid subunit (C_1–C_5) of integramycin (**47**), IUPAC numbering designations are *not* utilised. For clarity, the numbering system of the parent compound, integramycin, (**47**) is utilised consistently throughout.



lithospermic acid (**21**)



integramycin (**47**)