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# **Synthesis of Cyclodextrin Composites Incorporating Targeting and Drug Carrying Capabilities**

A thesis submitted in partial fulfilment of the  
requirements for the degree of

Doctor of Philosophy  
in Chemistry

Massey University, Turitea Campus,  
Palmerston North, New Zealand

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**2010**





# ABSTRACT

## Synthesis of Cyclodextrin Composites Incorporating Targeting and Drug Carrying Capabilities

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A selective, versatile, and robust methodology for the bi-functionalisation of  $\beta$ -cyclodextrin has been developed which allows for the attachment of peptides and/or sulfonamides in varying C- and N-terminal combinations on resin using N-Fluorenylmethoxycarbonyl (Fmoc) Solid Phase Peptide Synthesis (SPPS). Mono-6<sup>A</sup>-fluorenylmethoxycarbonylamino-mono-6<sup>X</sup>-succinyl- $\beta$ -cyclodextrin, an amino acid based bi-functionalised derivative of  $\beta$ -cyclodextrin, has been functionalised with the bioactive peptide, bradykinin, and/or sulfonamides using Fmoc SPPS on Rink resin. The all-in-one molecule contains a carrier (cyclodextrin), targeting agent (bradykinin) and/or drug (sulfonamide). Varying combinations of these bradykinin-focused molecules have been synthesised in an attempt to determine the structure-function relationship against cancer cell lines using cell-based screening *in vitro*.

This study commenced with the synthesis of two linkers on to cyclodextrin. This enabled selective binding directly on to the resin, or a peptide attached to the resin. Peptide growth and/or cleavage from the resin followed allowing for the synthesis of peptide-cyclodextrin species in various combinations. Fmoc SPPS techniques have been employed to allow for the addition and synthetic extension of peptides on to cyclodextrin. Peptide purification was achieved by reverse phase high pressure liquid chromatography, and nuclear magnetic resonance spectroscopy and mass spectrometry were used to determine the success of the coupling reactions and identification of cyclodextrin regio isomers. Sulfonamide additions to the cyclodextrin and/or peptide

compounds were obtained after numerous studies investigating the optimal reaction conditions. 4-Fluorenylmethyloxycarbonylaminobenzenesulfonyl chloride was found to give the highest yields for the synthesis of C-terminal peptide sulfonamides with 4-carboxybenzenesulfonamide giving the highest optimal yields for N-terminal peptide sulfonamides. Peptide coupling efficiency of cyclodextrin and sulfonamides were investigated and optimised by comparing different SPPS resins and solvents. The incorporation of spacers between the peptide/cyclodextrin and/or resin have also been investigated in an attempt to improve overall reaction yields.

Preliminary bioassay testing against tumour cell lines HT-29 Human Duodenum, Hs700T Human pancreatic adenocarcinoma, and MA-104 Human pancreatic adenocarcinoma were performed. The MTT assay and the flow cytometry assay were used to show the effect of varying combinations of these cyclodextrin-peptide-sulfonamide molecules against the three cell lines and compared to a known anticancer drug, 5-Fluorouracil. Despite employment of simple entities in the construction of these compounds, an increase in cell proliferation (*ca* 10-20%) was seen for some cyclodextrin-bradykinin complexes. In addition, an exposed C-terminus on the bradykinin-sulfonamide moiety and an exposed N-terminus on the cyclodextrin-bradykinin sulfonamide moiety both gave positive results. Mixed results were obtained with the addition of a linker between the cyclodextrin and the bradykinin molecules (less than 5% increase or decrease) compared to their non-linker counterparts.

*Part of this thesis has been published in:*

White, R. J., Plieger, P. G., and Harding, D. R. K., Synthesis of bi-functional peptide derivatives based on a  $\beta$ -cyclodextrin core with drug delivery potential. *Tetrahedron Letters* **2009**, 51 (5), 800-803.

## ACKNOWLEDGEMENTS

I would like to especially thank my supervisors Associate Professor David R. K. Harding and Dr Paul G. Plieger for their outstanding advice, guidance, patience, and support throughout the length of this project. I would also like to thank David Lun for the distillation of various solvents, his help on some synthetic work, and for his assistance with the initial training on the HPLC, MALDI and ESI-MS. The knowledge gained from him regarding the use and maintenance of lab equipment has also helped me to understand aspects of this project better and has given me skills that will be valuable for the future. These include the full care and upkeep of the MALDI, ESI-MS, freeze dryers, water Milli-Q purification system, as well as HPLC upkeep and HPLC column maintenance. It has been very much appreciated.

I also wish to thank Dr Marco Wenzel for collecting the x-ray data on my crystals and Dr Paul Plieger for his crystal growing advice and solving the X-ray data sets. I would also like to acknowledge Dr Pat Edwards for his help when using the NMR equipment including running samples on the 700 MHz NMR and advice for structural analysis of some compounds, the Centre for Separation Science laboratory (at Massey University), and to the many people in the Department of Chemistry who have helped me along the way. I would like to express my appreciation to Fran Wolber and her team from the Institute of Food Nutrition and Human Health at Massey University for carrying out bioassay analysis on my samples and the help and advice she has given regarding bioassays in general.

I also wish to extend my gratitude to Massey University and the Royal Society of Chemistry for the funding which has supported my work recorded here at the Centre of Separation Science, Massey University, Palmerston North, 2010 and travel to conferences abroad.

Finally, I wish to extend a big thank you to my family and friends for their continuous support throughout this project, without their endless support and encouragement, it would have never come to fruition.

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## LIST OF ABBREVIATIONS AND SYMBOLS

<b>AA</b>	Amino acid
<b>Ala</b>	Alanine (amino acid)
<b>aq</b>	Aqueous
<b>Arg</b>	Arginine (amino acid)
<b>β-CD</b>	Beta-cyclodextrin
<b>B<sub>1</sub>, B<sub>2</sub></b>	Bradykinin receptors
<b>Bi-</b>	Two units added
<b>BK</b>	Bradykinin
<b>Boc</b>	<i>Tert</i> -butyloxycarbonyl
<b>BOP</b>	1-Benzotriazolyl-oxy-tris-dimethylamino-phosphonium-hexafluorophosphate
<b>br</b>	Broad (spectral)
<b>Bn-</b>	Benzyl ester (protecting group)
<b><sup>13</sup>C NMR</b>	Carbon. In reference to NMR spectroscopy utilising the <sup>13</sup> C isotope of carbon
<b>2ClZ</b>	2-Chloro-Z, <i>N</i> -(2-Chlorobenzoyloxycarbonyloxy)
<b>C-18</b>	HPLC column coated with a carbon 18 reverse phase
<b>CA</b>	Carbonic anhydrase
<b>Cbz</b>	Carbobenzoyloxy (protecting group)
<b>Cbz-OSu</b>	<i>N</i> -α-(Benzyloxycarbonyloxy) succinimide
<b>CD</b>	Cyclodextrin
<b>CD OH<sub>2</sub>, OH<sub>3</sub></b>	Cyclodextrin secondary hydroxy groups labelling
<b>CD C1-C6</b>	Carbon labelling for an amylose ring
<b>cm</b>	Centimetres
<b>COSY</b>	Correlation spectroscopy
<b>C-terminus</b>	Carboxy terminal (of a peptide)
<b>Cys</b>	Cysteine (amino acid)
<b>4-DMAP</b>	4-Dimethylaminopyridine
<b>d<sub>6</sub>-DMSO</b>	Dimethyl-d <sub>6</sub> sulfoxide
<b>d</b>	Doublet (spectral)























<b>D-</b>	D-optical isomer (of amino acids)
<b>Da</b>	Daltons
<b>DBU</b>	1,8-Diazabicyclo[5.4.0]undec-7-ene
<b>DCC</b>	<i>N,N'</i> -Dicyclohexylcarbodiimide
<b>De-Boc</b>	Removal of the <i>tert</i> -butyloxycarbonyl protecting group
<b>DEPT</b>	Distortionless enhancement by polarization transfer
<b>Des</b>	One less of something
<b>Dhbt</b>	3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
<b>DHPS</b>	Dihydropteroate synthase
<b>DIEA</b>	<i>N,N</i> -Diisopropylethylamine (Hunig's base)
<b>DIPCD</b>	<i>N,N'</i> -Diisopropylcarbodiimide
<b>DMEM</b>	Delbecco's modified eagle's medium
<b>E7070</b>	Sulfonamide
<b>ECM</b>	Extracellular matrix
<b>EDC</b>	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
<b>EDT</b>	Ethanedithiol
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>Endo-</b>	Internal functionalisation
<b>EPR</b>	Enhanced Permeability and Retention factor
<b>equiv</b>	Equivalent(s)
<b>ESI-MS, ESI</b>	Electrospray Ionisation Mass Spectrometry
<b>Exo-</b>	External functionalisation
<b>5-FU</b>	5-Fluorouracil
<b>FCS</b>	Foetal calf serum
<b>Fmoc</b>	<i>N</i> -Fluorenylmethoxycarbonyl
<b>Fmoc-OSu</b>	<i>N</i> -(9-Fluorenylmethoxycarbonyloxy) Succinimide
<b>G0/G1</b>	Cell cycle phase(s)
<b>G1</b>	Cell cycle phase
<b>G2</b>	Cell cycle phase
<b>G2/M</b>	Cell cycle phase(s)
<b>g</b>	Gram(s)
<b>Gly</b>	Glycine (amino acid)
<b>g/mol</b>	Grams per mole

<b><sup>1</sup>H NMR</b>	Proton (hydrogen). In reference to NMR spectroscopy utilising the <sup>1</sup> H isotope of hydrogen
<b>H<sup>+</sup></b>	Proton
<b>h</b>	Hour(s)
<b>HATU</b>	2-(6-Aza-1-H- Benzotriazole-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
<b>HBTU</b>	O-Benzotriazole- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
<b>HCTU</b>	2-(6-Chloro-1-H-Benzotriazole-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
<b>Hexa-</b>	Six units added
<b>His</b>	Histidine (amino acid)
<b>HMP resin</b>	4-Hydroxymethylphenoxyacetyl-4'-methylbenzydryl-amine resin (Wang resin)
<b>HOBT</b>	Hydroxybenzotriazole
<b>HPLC</b>	High pressure liquid chromatography
<b>HR-MS</b>	High resolution mass spectrometry
<b>Hs700T</b>	Cancer cell line
<b>HT-29</b>	Cancer cell line
<b>Hz</b>	Hertz
<b>IFNHH</b>	Institute of Food Nutrition and Human Health
<b>Igl</b>	$\alpha$ -Amino-2-indanacetic acid
<b>Ile</b>	Isoleucine (amino acid)
<b><i>In situ</i></b>	In the same place
<b><i>in vacuo</i></b>	In vacuum
<b>IR</b>	Infrared spectroscopy
<b><i>J</i></b>	Coupling constant (in NMR spectrometry)
<b>K</b>	Kilo
<b>K-562</b>	Cancer cell line
<b>K<sup>+</sup></b>	Potassium ions
<b>L-</b>	L-optical isomer (of amino acids)
<b>Leu</b>	Leucine (amino acid)
<b>Linker</b>	Joins the resin to the functional group in solid phase peptide synthesis

<b>l/h</b>	Litres per hour
<b>LR-MS</b>	Low Resolution Mass Spectrometry
<b>m</b>	Multiplet (spectral)
<b>M</b>	Cell cycle phase
<b>M<sup>+</sup></b>	Parent molecular ion
<b>MA-104</b>	Cancer cell line
<b>MALDI-TOF</b>	Matrix assisted laser desorption ionisation time-of-flight
<b>MBHA</b>	<i>p</i> -Methyl-benzylhydramine
<b>MCF-7</b>	Cancer cell line
<b>Met</b>	Methionine, amino acid
<b>mg</b>	Milligram
<b>MHz</b>	Megahertz
<b>min</b>	Minute(s)
<b>mL</b>	Millilitre(s)
<b>mL/min</b>	Millilitre per minute
<b>mmol</b>	Millimole
<b>MMP</b>	Matrix metalloproteinases
<b>mol</b>	Mole(s)
<b>mol/L</b>	Moles per litre
<b>Mono-</b>	Single, one unit added
<b>MS</b>	Mass spectrometry
<b>Mtr</b>	4-Methoxy-2,3,6-trimethylbenzenesulfonyl group
<b>MTT</b>	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
<b>m/z</b>	Mass-to-charge ratio
<b>Na<sup>+</sup></b>	Sodium ions
<b>nm</b>	Nanometre(s)
<b>NMM</b>	<i>N</i> -Methylmorpholine
<b>NMR</b>	Nuclear magnetic resonance
<b>NSCLC</b>	Non-small cell lung carcinoma
<b><i>N</i>-terminus</b>	Amide terminal (of a peptide)
<b>Nu<sup>-</sup></b>	Nucleophile
<b>OBt</b>	Oxy-benzotriazole (active ester)
<b>OC</b>	Oxalyl chloride

<b>OD</b>	Optical density
<b>PABA</b>	Para-aminobenzoic acid
<b>PAM resin</b>	4-Hydroxymethylphenylacetamidomethyl resin
<b>Pbf</b>	2,2,7,7,8-Pentamethylchromane-6-sulfonyl
<b>Pd/C</b>	Palladium on carbon (catalyst)
<b>PEG</b>	Poly(ethylene glycol)
<b>PEGA-based support</b>	PEG copolymers, e.g. bis 2-acrylamino-2-propyl-poly-ethylene glycol
<b>Pfp</b>	Pentafluorophenylesters
<b>Phe</b>	Phenylalanine (amino acid)
<b>PI</b>	Propidium iodide
<b>Pip</b>	Piperidine
<b>ppm</b>	Parts per million
<b>Pro</b>	Proline (amino acid)
<b>PS</b>	Polystyrene
<b>PSA</b>	Preformed symmetrical anhydrides
<b>PSG</b>	Penicillin-streptomycin-L-glutamine
<b>Psi</b>	Pounds per square inch
<b>PyBOP</b>	1-Benzotriazolyl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
<b>PyBroP</b>	Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
<b>q</b>	Quartet (spectral)
<b>rbf</b>	Round bottom flask(s)
<b>R<sub>f</sub></b>	Retention factor (in chromatography)
<b>RP-</b>	Reverse phase
<b>rpm</b>	Revolutions per minute
<b>t<sub>R</sub></b>	Retention time (in chromatography)
<b>rt</b>	Room temperature
<b>s</b>	Singlet (spectral)
<b>S</b>	Cell cycle phase
<b>S/G2/M</b>	Cell phases of a cell cycle
<b>SCLC</b>	Small cell lung carcinoma
<b>Sec</b>	Second(s)

<b>SEM</b>	Standard error of the mean
<b>Ser</b>	Serine (amino acid)
<b>Spacer</b>	Moiety used to separate various components in cyclodextrin/sulfonamide/peptide complexes
<b>SPPS</b>	Solid phase peptide synthesis
<b>t</b>	Triplet (spectral)
<b><i>t</i>-butyl, <i>t</i>-Bu</b>	Tertiary-butyl group
<b>TBTU</b>	O-Benzotriazol-1-yl- <i>N,N,N'</i> -tetra-methyluronium tetrafluoroborate
<b>TCFC</b>	Tetramethyl chloro formamidinium hexachlorophosphate
<b>TCFH</b>	Tetramethyl chloro formamidinium hexafluorophosphate
<b>TEA</b>	Triethylamine
<b>TFA</b>	Trifluoroacetic acid
<b>TFFH</b>	Tetramethyl fluoro formamidinium hexafluorophosphate
<b>TFMSA</b>	Trifluoromethanesulfonic acid
<b>THC</b>	Tetrahydrocannabinol
<b>Thr</b>	Threonine, amino acid
<b>TLC</b>	Thin-layer chromatography
<b>TMS</b>	Trimethylsilyl
<b>TMU</b>	Tetramethyl urea
<b>TNBS</b>	2,4,6-Trinitrobenzene sulfonic acid
<b>TOF</b>	Time-of-flight
<b>Tri-</b>	Three units added
<b>Trp</b>	Tryptophan (amino acid)
<b>Ts, Tos</b>	<i>N</i> -Tosyl
<b>Tyr</b>	Tyrosine (amino acid)
<b>UV-Vis</b>	Ultraviolet-visible spectroscopy
<b>V</b>	Voltage
<b>Val</b>	Valine (amino acid)
<b>WSCD</b>	Water soluble carbodiimide
<b>XXX<sub>1</sub></b>	Refers to the position of the amino acid in a peptide chain
<b>6<sup>A</sup>-</b>	Number location for the attachment of functional groups onto cyclodextrins primary hydroxy groups

$6^X$ -	Unknown positional attachment to cyclodextrins primary hydroxy groups
$^{\circ}\text{C}$	Degrees centigrade
$\mu\text{L}$	Microlitre(s)
$\mu\text{g}$	Microgram(s)
$\mu\text{mol}$	Micromolar(s)
$\mu\text{g/mL}$	Microgram per millilitre
$\mu\text{L/min}$	Microlitre per minute
$\gamma$ -	Gamma
$\delta$	Chemical Shift in parts per million
	Spacer ( $\epsilon$ -aminocaproic acid)
	Rink amide resin
	TentaGel resin
	PAM resin
	Rink-spacer resin
	Peptide(s)
	Sieber resin
	Sulfonamide replacement for PABA
	Polymer support
	Side-chain protecting group
	N- $\alpha$ -protecting group
	Activating group(s)
	Para-amino-benzoic acid (PABA)
	Enzyme(s)
	Glutamic acid substrate
	Folic acid
	N- and/or C-terminal peptide
	Protecting group(s)
	C-terminal sulfonamide, 4-aminobenzenesulfonyl chloride
	N-terminal sulfonamide, 4-carboxybenzenesulfonamide
	Bradykinin peptide
	Val-Gly-Ala (tri-peptide)