Synthesis of Cyclodextrin Composites Incorporating Targeting and Drug Carrying Capabilities

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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 β-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^{A}-fluorenylmethyloxycarbonylamino-mono-6^{X}-succinyl-β-cyclodextrin, an amino acid based bi-functionalised derivative of β-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 then 5% increase or decrease) compared to their non-linker counterparts.

Part of this thesis has been published in:

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	Amino acid
Ala	Alanine (amino acid)
aq	Aqueous
Arg	Arginine (amino acid)
β-CD	Beta-cyclodextrin
B_1, B_2	Bradykinin receptors
Bi-	Two units added
BK	Bradykinin
Boc	*Tert*-butyloxycarbonyl
BOP	l-Benzotriazolyloxy-tris-dimethylamino-phosphonium-hexafluorophosphate
br	Broad (spectral)
Bn-	Benzyl ester (protecting group)
^{13}C NMR	Carbon. In reference to NMR spectroscopy utilising the ^{13}C isotope of carbon
2ClZ	2-Chloro-Z, *N*-(2-Chlorobenzyloxycarbonyloxy)
C-18	HPLC column coated with a carbon 18 reverse phase
CA	Carbonic anhydrase
Cbz	Carbobenzyloxy (protecting group)
Cbz-OSu	*N*-α-(Benzyloxycarbonyloxy) succinimide
CD	Cyclodextrin
CD OH2, OH3	Cyclodextrin secondary hydroxy groups labelling
CD C1-C6	Carbon labelling for an amylose ring
cm	Centimetres
COSY	Correlation spectroscopy
***C*-terminus**	Carboxy terminal (of a peptide)
Cys	Cysteine (amino acid)
4-DMAP	4-Dimethylaminopyridine
d_6-DMSO	Dimethyl-d_6 sulfoxide
d	Doublet (spectral)

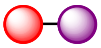
D-	D-optical isomer (of amino acids)
Da	Daltons
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-Dicyclohexylcarbodiimide
De-Boc	Removal of the *tert*-butyloxycarbonyl protecting group
DEPT	Distortionless enhancement by polarization transfer
Des	One less of something
Dhbt	3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine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

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

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

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

SEM	Standard error of the mean
Ser	Serine (amino acid)
Spacer	Moiety used to separate various components in cyclodextrin/sulfonamide/peptide complexes
SPPS	Solid phase peptide synthesis
t	Triplet (spectral)
***t*-butyl, *t*-Bu**	Tertiary-butyl group
TBTU	O-Benzotriazol-1-yl-*N,N,N'*-tetra-methyluronium tetrafluoroborate
TCFC	Tetramethyl chloro formamidinium hexachlorophosphate
TCFH	Tetramethyl chloro formamidinium hexafluorophosphate
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFFH	Tetramethyl fluoro formamidinium hexafluorophosphate
TFMSA	Trifluoromethanesulfonic acid
THC	Tetrahydrocannabinol
Thr	Threonine, amino acid
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TMU	Tetramethyl urea
TNBS	2,4,6-Trinitrobenzene sulfonic acid
TOF	Time-of-flight
Tri-	Three units added
Trp	Tryptophan (amino acid)
Ts, Tos	*N*-Tosyl
Tyr	Tyrosine (amino acid)
UV-Vis	Ultraviolet-visible spectroscopy
V	Voltage
Val	Valine (amino acid)
WSCD	Water soluble carbodiimide
XXX_1	Refers to the position of the amino acid in a peptide chain
6^A-	Number location for the attachment of functional groups onto cyclodextrins primary hydroxy groups

6^{X}-	Unknown positional attachment to cyclodextrins primary hydroxy groups
°C	Degrees centigrade
µL	Microlitre(s)
µg	Microgram(s)
µmol	Micromolar(s)
µg/mL	Microgram per millilitre
µL/min	Microlitre per minute
γ-	Gamma
δ	Chemical Shift in parts per million
	Spacer (ε-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-α-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-carboxyenzenesulfonamide
	Bradykinin peptide
	Val-Gly-Ala (tri-peptide)