CHEMCOMM
Communication
www.rsc.org/chemcomm

β-Cyclodextrin dimers as potential tumor pretargeting agents†‡

W. Barry Edwards,[a] David E. Reichert,[a] D. André d'Avignon[b] and Michael J. Welch*[a]

[a] *Mallinckrodt Institute of Radiology, Washington University School of Medicine, 510 South Kingshighway Boulevard, St. Louis, MO 63110, USA. E-mail: welchm@mir.wustl.edu*
[b] *Department of Chemistry, Washington University, St. Louis, MO 63110, USA*

Received (in Columbia, MO, USA) 27th March 2001, Accepted 1st June 2001
First published as an Advance Article on the web 27th June 2001

A β-cyclodextrin dimer binds a di-*tert*-butylbenzyl–Cu–cyclen with high affinity, demonstrating potential as a receptor/ligand system for tumor pretargeting with monoclonal antibodies.

Monoclonal antibodies (Mabs) have advantages for the delivery of a radioisotope (or toxin) to tumor sites due to their high affinity and specificity for their antigen.[1,2] However, the results of radioimmunotherapy of solid tumors have been disappointing because radiolabeled Mabs targeted to cell surface antigens impart a high radiation dose to normal organs causing toxicity.[3] In part, this is due to the long circulating times for Mabs.

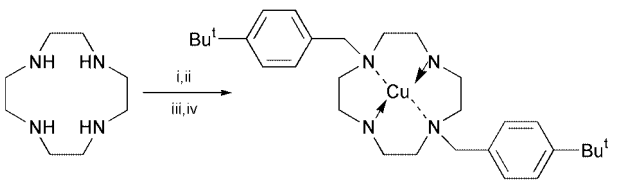
Successful imaging or therapy with radiolabeled Mabs depends not only on the concentration of the Mab at the targeted site but on clearance rates from normal tissues relative to that from the tumor. Imaging studies in humans have shown that maximum concentrations of Mabs at the tumor site are attainable within 24 h, but several more days are required before the concentration of the radiolabeled Mab in the circulation decreases to levels low enough for successful imaging to take place.[4] Pretargeting techniques seek to maintain high accumulation of the radionuclide at the target site while minimizing non-target tissue toxicity (see reviews[1,2]). One current approach at pretargeting utilizes a Mab conjugated to a receptor with a high affinity for a ligand bearing the radionuclide. The most often utilized receptor/ligand system is avidin (or streptavidin/biotin).[5] After the Mab-receptor conjugate has localized at the tumor site, a low molecular weight radiolabeled ligand, one that is rapidly excreted *via* the kidneys, is administered to visualize the tumor. To be effective, the ligand must be rapidly excreted from the body to provide the desired high tumor accumulation with relatively low non-target accumulation.

An alternative receptor for tumor pretargeting could be a β-cyclodextrin dimer. Breslow *et al.* studied a series of β-cyclodextrin dimers and achieved some of the highest affinities reported to date.[6,7] While the best match between a guest molecule and single cyclodextrin subunit will provide affinities on the order of 10^{-4} M, cyclodextrin dimers can increase affinities to 10^{-8} to 10^{-10} M.[8] With affinities of this magnitude, β-cyclodextrin dimers could serve as receptors for tumor pretargeting. A potential strategy for pretargeting is the development of a radiometal-binding macrocycle containing pendant hydrophobic groups with the appropriate geometry for inclusion into the cavity of the cyclodextrins. Ultimately, the β-cyclodextrin dimer would be concentrated at the tumor site by conjugation *via* a covalent bond to a Mab. Radiometals such Cu-64 [$t_{1/2}$ = 12.7 h; $E_{\beta max}$ = 0.653 MeV (17.4%); $E_{\beta max}$ = 0.573 MeV (40%)] and Cu-67 [$t_{1/2}$ = 62 h; $E_{\beta max}$ = 0.392 MeV (56%)] have been used previously in imaging[9] and therapy[10,11] of tumors. To demonstrate the feasibility of this new approach, Cu–BBC (Scheme 1) and a β-cyclodextrin dimer (Scheme 2) were synthesized and affinities determined in an equilibrium binding assay. A fluorescent dye, BNS, was synthesized as previously described.[12]

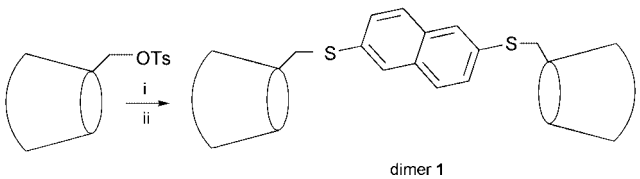
Breslow *et al.* reported binding affinities for a β-cyclodextrin dimer with a single linkage in the low nanomolar region for substrates bearing two *tert*-butylphenyl substituents.[6] Therefore, a β-cyclodextrin dimer with a single linkage, was synthesized to serve as a host for a copper chelate-complex bearing two *tert*-butylphenyl substituents. The dimer **1** was prepared from commercially available tosyl-β-cyclodextrin and 2,6-naphthalene dithiol[13] (Scheme 2). The cyclodextrin dimer was purified by HILIC then by silica-gel chromatography.[14] Analysis by ^{1}H, ^{13}C, HMQC, COSY NMR and MALDI-MS confirmed the dimeric structure of the cyclodextrin.

Cyclen was chosen as the chelator because of its high stability (log K_f = 24.6) for copper(II).[15] Based on synthetic methodology for selectively protecting the 1 and 7 nitrogens of tetraazamacrocycles,[16] alkylation of the nitrogens with commercially available *tert*-butylbenzyl bromide appeared to be the most expedient way of introducing a pair of hydrophobic substituents onto the chosen chelator (Scheme 1). Molecular modeling showed that a distance of *ca.* 16 Å is attainable between the two tertiary carbons of the *tert*-butyl groups and between the midpoints of the β-cyclodextrin subunits of dimer **1** when the faces containing the primary hydroxyl groups are opposed to each other.[17]

Equimolar amounts of copper(II) acetate and BBC resulted in the quantitative formation of Cu–BBC. While Cu–BBC and BBC co-eluted on a C-18 column, a diphenyl column resolved a co-injected mixture of the two. Analysis of the reaction



Scheme 1 *Reagents and conditions*: i, pH 2.5, water, chloroethylformate; ii, *tert*-butylbenzyl bromide, DMF; iii, hydrazine, ethylene glycol, KOH; iv, copper(II) acetate, aq. NH_4O_2CMe, pH 6.4.



Scheme 2 *Reagents and conditions*: i, KI, DMF, 85 °C; ii, 2,6-naphthalenedithiol, NH_4HCO_3, DMF, 85 °C.

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b1/b102814f/

‡ Abbreviations: Mabs, monoclonal antibodies; HILIC, hydrophilic interaction chromatography; HMQC, heteronuclear multiple quantam coherence; COSY, H–H correlated spectroscopy; MALDI-MS, matrix assisted laser desorption-ionization mass spectrometry; K_f, formation constant; BBC, 1,7-(4-*tert*-butylphenylmethyl)cyclen; Cu–BBC, copper-1,7-(4-*tert*-butylphenylmethyl)cyclen; ES-MS, electrospray mass spectrometry; HPLC, high pressure liquid chromatography; BNS, 6-(4-*tert*-butylphenylamino)naphthalene-2-sulfonic acid; K_D, equilibrium dissociation constant; K_i, the concentration of the competing ligand that will bind to half the binding sites at equilibrium; IC_{50}, concentration of competitive ligand that inhibits half of the binding of a ligand; Cheng–Prusoff equation, $K_i = IC_{50}(1 + [ligand]/K_{D,ligand})^{-1}$

 DOI: 10.1039/b102814f

This journal is © The Royal Society of Chemistry 2001

Table 1 Affinities of BNS and Cu–BBC for β-cyclodextrin and dimer **1**

Guest	Host	Calculated affinities	95% CI
BNS	β-Cyclodextrin	K_{D1} = 14 μM	10–18 μM
		K_{D2} = 1.6 mM	0.6–2.6 mM
BNS	Dimer **1**	K_D = 388 nM	112–664 nM
Cu–BBC[a]	Dimer **1**	K_i = 18 nM	12–28 nM
		IC_{50} = 120 nM	76–189 nM

[a] BNS was included. Cu–BBC was a competitive ligand with varying concentration.

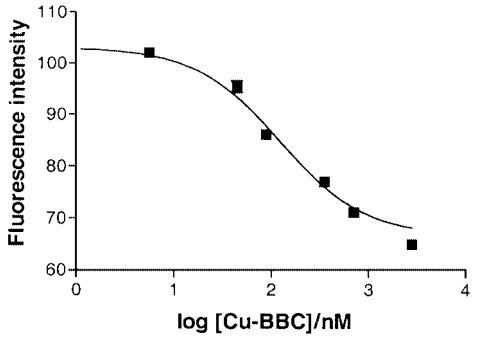


Fig. 1 Heterologous competitive binding assay between Cu–BBC and BNS for dimer **1**.

mixture on the diphenyl column showed that BBC accounted for < 1% of the total peak area observed (λ = 215 nm, Cu–BBC and BBCs strongest absorbance). Analysis by ES-MS confirmed that the component observed by HPLC was Cu–BBC.

Affinity constants for β-cyclodextrin dimers and their substrates are often ascertained by fluorescence spectroscopy§ since saturation binding will be attained at concentrations too low to utilize NMR and UV spectroscopy. Therefore a fluorescent dye, BNS, was synthesized. BNS fluoresces weakly in water, but its inclusion into a hydrophobic environment, such as that of the cavity of β-cyclodextrin, enhances its fluorescence. This dye has been used extensively in the characterization of other β-cyclodextrin dimers (see review[8]). Curve fitting with commercially available software will then yield the affinity of dye. After the affinity of dye for its host has been quantified, a heterologous competitive binding assay between the dye and another guest can provide the affinity of the guest. These approaches were taken to evaluate the affinity of BNS and Cu–BBC for dimer 1 (Table 1).

BNS exhibited an enhancement of fluorescence in aqueous solution when interacting with the β-cyclodextrin subunits. The interaction was characteristic of a receptor–ligand (host–guest) complex because of its saturable and reversible nature. The observed affinity of BNS agrees with the previously determined value of K_D = 20 μM.[12] Previous affinities of BNS observed for β-cyclodextrin dimers with a single linkage have ranged from *ca.* 3 μM to 100 nM.[18,19] The increase in affinity of BNS is consistent with previously observed increases when two β-cyclodextrin subunits are linked face to face.

Because the fluorescence of Cu–BBC is unaffected by the presence of dimer **1**, its affinity must be determined by competition with BNS. The log-dose dependent displacement of BNS from dimer **1** with Cu–BBC indicates that both Cu–BBC and BNS have formed a complex with dimer **1** (Fig. 1). By substituting the value of the IC_{50} of Cu–BBC and the K_D of BNS into the Cheng–Prussof equation, the K_i of Cu–BBC is obtained.[20] This remarkably strong affinity bodes well for the success of dimer **1** and Cu–BBC as a receptor/ligand system for tumor pretargeting.

In conclusion, a β-cyclodextrin dimer with a naphthalene linker, dimer **1**, was synthesized for evaluation as the receptor in a new approach to tumor pretargeting. Dimer **1** showed a higher affinity for a fluorescent dye, BNS, than did β-cyclodextrin. BBC, a derivative of cyclen, was synthesized and Cu–BBC was prepared. Cu–BBC was strongly bound to dimer **1** (K_i = 18 nM relative to BNS). With affinities in the low nanomolar region, Cu-64–BBC and dimer **1** could serve as a receptor/ligand system for tumor pretargeting.

This work was supported by the United States Department of Energy (DE-FG02-87ER60512).

Notes and references

§ *Determination of affinity constants*: Utilizing GraphPad Prism® software (GraphPad Software Inc., San Diego, CA), the data were fit by non-linear regression to: $F = (F_{max})[BNS]/(K_D + [BNS])$ where F is the observed fluorescence intensity generated from incorporation of BNS into the hydrophobic interior of β-cyclodextrin (corrected for background fluorescence from unincorporated BNS or dimer **1**) to determine values for K_D.[21,22] To determine the IC_{50} value, the data were fit by non-linear regression to: $F_{obs} = T + (T - B)/1 + 10 \exp(\log[Cu–BBC] - \log IC_{50})$, where T and B are the top and bottom plateaus of the fitted curve and F_{obs} is the observed fluorescence.[21,22] To calculate K_i for Cu–BBC, the value obtained for the K_D of BNS and Cheng–Prussof equation were utilized.[20–22] The data were fit to both one or two binding sites in all experiments and the results for two binding sites are reported when $P < 0.05$. The error is represented in terms of 95% confidence interval (95% CI).

1 D. A. Goodwin and C. F. Meares, *Cancer Biother. Radiopharm.*, 1999, **14**, 145.
2 J. Barbet, F. Kraeber-Bodere, J. Vuillez; E. Gautherot, E. Rouvier and J. Chatal, *Cancer Biother. Radiopharm.*, 1999, **14**, 153.
3 R. B. Wilder, G. L. DeNardo and S. J. DeNardo, *J. Clin. Oncol.*, 1996, **14**, 1383.
4 D. A. Goodwin, *J. Nucl. Med.*, 1995, **36**, 876.
5 D. B. Axworthy, P. L. Beaumier, B. J. Bottino, S. Goshorn, R. W. Mallet, K. M. Stone, F. M. Su, L. F. Theodore, E. K. Yau and J. M. Reno, *Tumor Targeting*, 1996, **2**, 156.
6 R. Breslow, N. Greenspoon, T. Guo and R. Zarzycki, *J. Am. Chem. Soc.*, 1989, **111**, 8296.
7 R. Breslow and S. Chung, *J. Am. Chem. Soc.*, 1990, **112**, 9659.
8 R. Breslow, S. Halfon and B. Zhang, *Tetrahedron*, 1995, **51**, 377.
9 C. J. Anderson, L. A. Jones, L. A. Bass, E. L. Sherman, D. W. McCarthy, P. D. Cutler, M. V. Lanahan, M. E. Cristel, J. S. Lewis and S. W. Schwarz, *J. Nucl. Med.*, 1998, **39**, 194.
10 C. J. Anderson, F. Dehdashti, P. D. Cutler, S. W. Schwarz, R. Laforest, L. R. Bass, J. S. Lewis and D. W. McCarthy, *J. Nucl. Med.*, 2001, **42**, 213.
11 G. R. Mirick, R. T. O'Donnell, S. J. DeNardo, S. Shen, C. F. Meares and G. L. DeNardo, *Nucl. Med. Biol.*, 1999, **26**, 841.
12 S. Chung, Ph.D. Thesis, Columbia University, 1991.
13 V. N. Lisitsyn, L. S. Kukalenko and A. M. Tsatkis, *Zh. Org. Khim.*, 1985, **21**, 161.
14 A. J. Alpert, *J. Chromatogr.*, 1990, **499**, 177.
15 R. M. Smith and A. E. Martell, *Crictical Stability Constants*, Plenum, New York, 1989.
16 Z. Kovacs and A. D. Sherry, *J. Chem. Soc., Chem. Commun.*, 1995, 185.
17 CambridgeSoft Corporation, CS Chem3D Std, 4.0, Cambridge MA, 1997.
18 R. Breslow and B. Zhang, *J. Am. Chem. Soc.*, 1996, **118**, 8495.
19 R. C. Petter, C. T. Sikorski and D. H. Waldeck, *J. Am. Chem. Soc.*, 1991, **113**, 2325.
20 Y. Cheng and W. H. Prusoff, *Biochem. Pharmacol.*, 1973, **22**, 3099.
21 L. E. Limbird, *Cell Surface Receptors: A short course on theory and methods*, Kluwer Academic Publishers, Hingham, 1986.
22 H. J. Molutsky, *Analyzing Data with GraphPad Prism*, GraphPad Software Inc., San Diego, 1999.