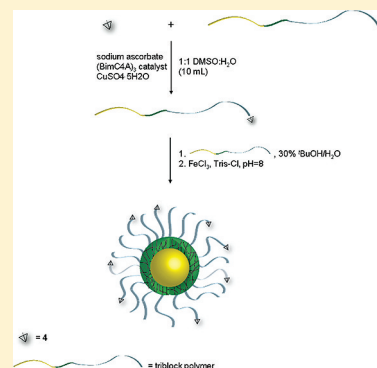


Development of Melanoma-Targeted Polymer Micelles by Conjugation of a Melanocortin 1 Receptor (MC1R) Specific Ligand

Natalie M. Barkey,[†] Narges K. Tafreshi,[†] Jatinder S. Josan,[‡] Channa R. De Silva,[§] Kevin N. Sill,^{||} Victor J. Hruby,^{‡,⊥} Robert J. Gillies,[†] David L. Morse,^{*,†} and Josef Vagner^{*,‡}[†]Department of Imaging, H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, Tampa, Florida, 33612, United States[‡]The BIO5 Research Institute, University of Arizona, 1657 E Helen Street, Tucson, Arizona 85721, United States[§]Department of Chemistry and Physics, Western Carolina University, Cullowhee, North Carolina 28723, United States^{||}Intezyne Technologies, Inc., 3720 Spectrum Boulevard, Suite 104, Tampa, Florida 33612, United States[⊥]Department of Chemistry and Biochemistry, University of Arizona, 1306 E. University Boulevard, Tucson, Arizona 85721, United States

Supporting Information

ABSTRACT: The incidence of malignant melanoma is rising faster than that of any other cancer in the United States. Because of its high expression on the surface of melanomas, MC1R has been investigated as a target for selective imaging and therapeutic agents against melanoma. Eight ligands were screened against cell lines engineered to overexpress MC1R, MC4R, or MC5R. Of these, compound 1 (4-phenylbutyryl-His-DPhe-Arg-Trp-NH₂) exhibited high (0.2 nM) binding affinity for MC1R and low (high nanomolar) affinities for MC4R and MC5R. Functionalization of the ligand at the C-terminus with an alkyne for use in Cu-catalyzed click chemistry was shown not to affect the binding affinity. Finally, formation of the targeted polymer, as well as the targeted micelle formulation, also resulted in constructs with low nanomolar binding affinity.



INTRODUCTION

The incidence of malignant melanoma is rising faster than that of any other cancer in the United States, with diagnoses having doubled from 1986 to 2001.¹ Melanoma progression is associated with altered expression of cell surface proteins, including adhesion proteins and receptors.² Over 80% of malignant melanomas express high levels of isoform 1 of the melanocyte stimulating hormone (α MSH) receptor (melanocortin 1 receptor, or MC1R).³ Thus, MC1R has been investigated as a target for selective imaging and therapeutic agents. MC1R belongs to a family of five G-protein-coupled melanocortin receptors (MC1R–MC5R). Melanocortin receptors have been discovered in a wide range of tissues and organs throughout the body, ranging from the hair/skin (MC1R),⁴ kidneys (MC5R),⁵ adrenal glands (MC2R),⁶ and hypothalamus (MC3R/MC4R)⁷ and are known to play a role in skin pigmentation, hair coloration, obesity, metabolism, diabetes, sexual behavior, erectile dysfunction, stress response, and mood.^{4–8} Endogenously, agonists for the melanocortins are the α -, β -, γ -melanocyte stimulating hormones (MSH) and adrenocorticotropic hormone (ACTH, MC2R specific), all of which contain the same central sequence of His-Phe-Arg-Trp.⁹ This high degree of pharmacophore homology makes it difficult

to design selective ligands that are highly specific for receptor subtypes.

Because of its high expression on the surface of melanomas, MC1R has been investigated as a target for selective imaging and therapeutic agents, and a number of selective ligands have been developed.¹⁰ The most well-known of these, [Nle⁴,D-Phe⁷]- α -MSH (NDP- α -MSH),¹¹ has been investigated extensively by Chen who showed that ^{99a}Tc-CGCG labeled NDP- α -MSH bound to melanomas with very high avidity (6.5% ID/g).¹² However, it is not selective, as NDP- α -MSH has strong nanomolar binding affinities to MC3R, MC4R, and MC5R as well.¹³ Such off-target binding is undesirable given the presence of these receptors in sensitive organs such as the kidney and brain. A co-injection of lysine has been reported to diminish off-target binding in the kidneys,^{12,14} and presumably most agents will not be able to cross the blood–brain barrier. Nonetheless, the need for the development of highly specific and selective ligands against MC1R for melanomas is of critical importance.

Received: July 11, 2011

Published: October 19, 2011

The development of ligands that can be attached to micelles and/or liposomes to target cancer cells relative to healthy organs is a major hurdle in current research. Stabilized micelles and liposomes are emerging as important platforms for delivery of lipophilic therapies to target tissues. Many such attempts fail from (1) a loss of affinity resulting from the attachment of small peptides to large micelles or liposomes, (2) an inherent instability that results in collapse before entering the vicinity of the tumor, or (3) a nanoparticle size that is too large to escape the vasculature. In order to effectively design targeted nanoparticles, each of these issues must be addressed.

In the current work, we screened eight putatively MC1R-specific compounds (Table 1) against cell lines that were

Table 1. Structures of Ligands Screened for MC1R Selectivity

compd	structure
1	4-phenylbutyryl-His-DPhe-Arg-Trp-NH ₂
2	Ac-homophenylalanine-His-DPhe-Arg-Trp-NH ₂
3	4-hydroxycinnamoyl-His-DPhe-Arg-Trp-NH ₂
4	4-phenylbutyryl-His-DPhe-Arg-Trp-Gly-Lys(hex-5-ynoyl)-NH ₂
5	H-Tyr-Val-Nle-Gly-His-DNal(2')-Arg-DTrp-Asp-Arg-Phe-Gly-NH ₂
6	H-Lys(hex-5-ynoyl)-Tyr-Val-Nle-Gly-His-DNal(2')-Arg-DTrp-Asp-Arg-Phe-Gly-NH ₂
7	H-Tyr-Val-Nle-Gly-His-DNal(2')-Arg-DPhe-Asp-Arg-Phe-Gly-NH ₂
8	H-Lys(hex-5-ynoyl)Tyr-Val-Nle-Gly-His-DNal(2')-Arg-DPhe-Asp-Arg-Phe-Gly-NH ₂

engineered to overexpress MC1R, MC4R, or MC5R, respectively. Compounds were tested for their ability to compete with Eu-NDP- α -MSH using a readout of time-resolved fluorescence (TRF).^{13b,15} Of these, compound 1 exhibited high binding affinity for MC1R and low affinities for MC4R and MC5R. Two analogues (compounds 2 and 3) have been synthesized to allow attachment via their N-termini; however, they exhibited a reduced (2) or complete loss (3) of binding affinity. Therefore, 1 was modified at the C-terminus (compound 4) to allow attachment to the micelle, and this modification was shown to not affect the binding affinity. Attachment to the stabilized polymer-based micelles was accomplished with Cu-catalyzed click chemistry, and the resulting construct was also shown to retain low nanomolar binding affinity.

RESULTS

Competitive Binding Assays. Competitive binding assays were performed using HCT116/hMC1R, HEK293/hMC4R, and HEK293/hMC5R cells with ligands 1–8. Seven of these were observed to bind to MC1R with low nanomolar affinity. Of these, only three related compounds (1, 2, and 4) were found to bind selectively to the hMC1R receptor, *vis-à-vis* MC4R or MC5R (Table 2). Compound 1 displayed slightly higher affinities for hMC1R compared to 2 (K_i is 0.17 and 1.77 nM for 1 and 2, respectively), as well as higher selectivity, especially compared to MC5R (selectivity ratios of 160 and 33, respectively). Ligands 5–8 demonstrated a high affinity for hMC1R as well; however, they were also shown to have an even stronger affinity for hMC4R and hMC5R (Table 2). Ligand 3 demonstrated no affinity for the hMC1R.

Competitive binding assays were also performed using 4-targeted triblock polymers, 4-targeted stabilized triblock polymer micelles, as well as untargeted polymer and untargeted,

Table 2. Affinity and Selectivity of Ligands Assayed in This Publication

ligand	K_i (nM)				
	MC1R	MC4R	MC5R	1R/4R	1R/5R
1	0.17	160	27	950	160
2	1.8	988	58	560	33
3	NB ^a	NB ^a	NB ^a		
4	0.24	254	46	1058	192
5	2.0	0.75	0.76	0.38	0.38
6	2.6	1.7	1.4	0.67	0.57
7	5.6	0.77	0.71	0.13	0.13
8	4.2	4.4	3.9	1.0	0.93
4-targeted polymer	26	NB ^a	NB ^a		
4-targeted micelles	2.9	NB ^a	NB ^a		
NDP- α -MSH	1.8	19	9.9	10	5.5

^aNB: nonbinding.

stabilized micelles as controls (Figure 1, Table 2). The 4-targeted micelle exhibits an increased binding avidity to the hMC1R receptor compared to the targeted polymer, and both are less avid than the native ligand (K_i of 2.9, 25.7, and 0.24 nM for the micelle, polymer, and ligand, respectively). No binding is observed with the untargeted polymer or untargeted micelles, indicating that the triblock polymer does not interact nonspecifically with the cell surface. Most notably, there is no measurable interaction ($\leq 10^{-5}$) between the 4-targeted micelle and hMC4R or hMC5R, indicating that the conjugation of the ligand to a micelle results in an increased selectivity.

Micelle Physical Properties. Dynamic light scattering (DLS) and zeta potential measurements showed the size and surface charge of the 4-targeted micelles to be 91 ± 2 nm and -10.6 ± 0.9 mV, respectively.

DISCUSSION

Historically, ligands that are known to interact with the hMC1R receptor also demonstrate cross-reactivity with other melanocortin receptors, including hMC4R and hMC5R. While MC1R is known to be expressed almost exclusively in melanoma cells and melanocytes, hMC4R and hMC5R have high expression levels in normal tissues, including kidney and brain; thus, nonspecific ligand binding is not ideal. To combat this problem and minimize off-target effects, we chose several ligands from the literature that have previously been reported to possess nanomolar binding affinities for hMC1R.

Ligand 1 was reported to have a high affinity and selectivity for MC1R (1R/4R selectivity ratio of 1200)^{10c,16} and was consequently chosen as a template for the design of the novel ligands 2 and 3, which contain the same parent amino acid sequence, as well as 4, which possesses a terminal alkyne. Ligands 2 and 3 were initially designed for potential attachment to a micelle through the N-terminus. Compound 2 represents the α -amino analogue of 1, and compound 3 possesses a more rigid C2 (cinnamate) aromatic linker compared to the 4-phenylbutyryl, 1, which has free rotation about the C3 chain. The 4-hydroxy group in compound 3 was intended as an attachment point to the micelles via O-alkylation. As mentioned, the rigid C2 linker in 3 reduces the free rotation of compound 1 and thus we reasoned might potentially result in improved interaction with the hydrophobic cleft of the MC1R. Unfortunately, compound 3 exhibited no affinity to the MC1R receptor, and compound 2 exhibited 10 times lower binding affinity compared to 1. Therefore, we chose to proceed

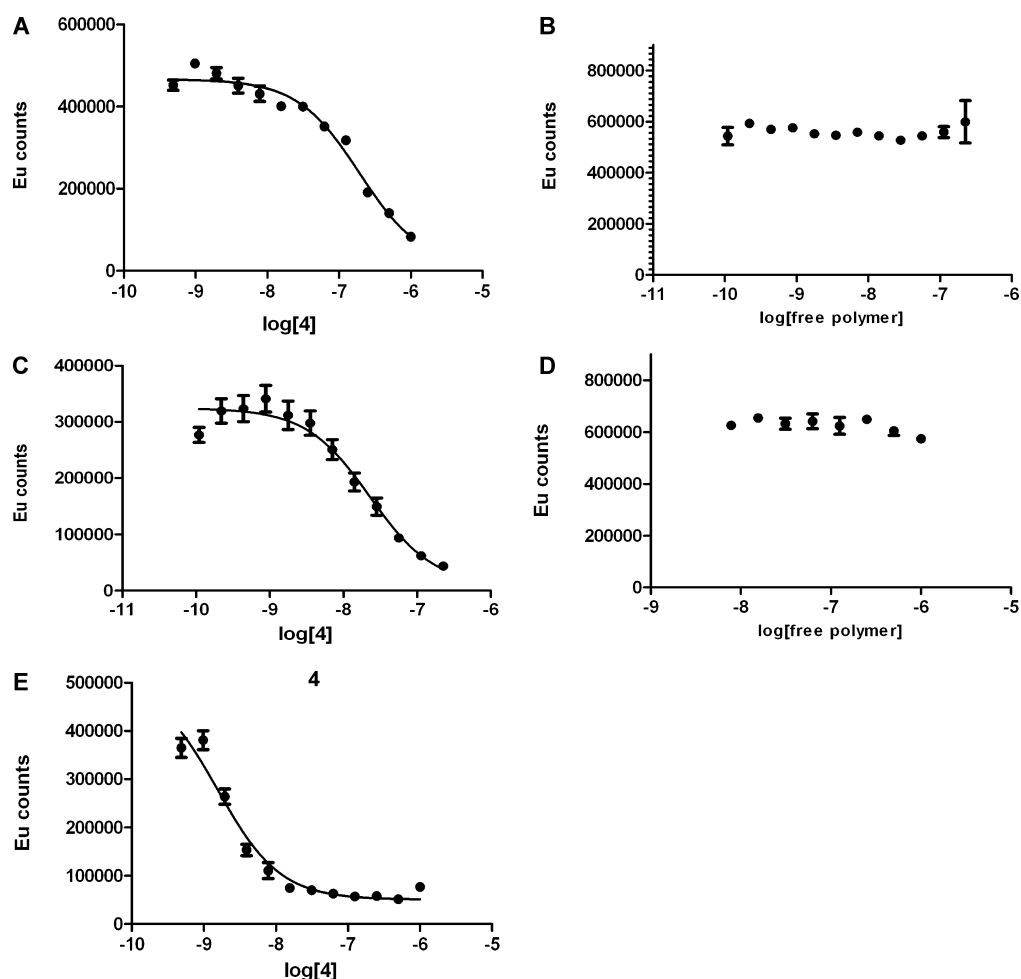


Figure 1. Representative competitive binding assays for (A) 4-targeted polymer, (B) untargeted polymer, (C) 4-targeted micelles, (D) untargeted micelles, and (E) 4 against HCT116/hMC1R cells. X-Axis concentrations for (A), (C), and (E) were normalized to the targeting ligand. Concentrations for the ligand in (A), (C), and (E) and polymer and micelle in (B) and (D) were deliberately chosen to be the same for all assays.

with a C-terminal modification (i.e., compound 4), as the binding affinity was not changed compared to the parent compound 1. Additionally, ligands 5 and 7 were reported to have moderate hMC1R selectivities over hMC4R and hMC5R (1R/4R of 20.00 and 12.50 nM for 5 and 7, respectively; 1R/5R of 11.67 and 2.06 for 5 and 7, respectively);^{13a} thus, each was functionalized with a terminal alkyne for attachment to a nanoparticle scaffold via click chemistry and screened for retained selectivity to MC1R.

The most specific of these ligands, 1, was determined by us to have an hMC1R/hMC4R selectivity of 950, which is in good agreement with the reported selectivity of 1200. Likewise, ligand 2, based on the same parent amino acid sequence as 1, was also found to have high 1R/4R selectivity; however, its 1R/5R selectivity was substantially lower. Unfortunately, ligands 3 and 5–8 were found to be not at all selective for MC1R, with ligand 3 possessing no affinity for any of the receptors tested. Our results for ligands 5 and 7 deviate from that which has been previously reported; however, this discrepancy may be due to differences in the binding assays used to derive the affinity constants. As detailed in the Experimental Section, our lab derived K_i values based on europium time-resolved fluorescence assays; however, previously determined EC_{50} values for these ligands were derived via ¹²⁵I-labeled competitive binding assays.

As 1 was determined to be the ligand with the highest hMC1R affinity and selectivity, it was chosen for modification with a terminal alkyne for attachment of a triblock polymer micelle. Compound 4 did not demonstrate a loss of affinity of MC1R following alkyne functionalization.

As predicted, 1 and 2 have similar binding profiles given the similarity in their structures; however, it was surprising to see a complete loss of affinity in 3. The differences in affinity among these three ligands arise from the structural differences at the N-terminal end of the peptide, given that they all share the same R-HfRW-NH₂ parent scaffold. However, whereas 1 and 2 contain Ph-(CH₂)₃-CO- and Ac-Hpe groups, both of which are nonpolar, at the N-terminus, 3 contains a 4-hydroxy-Ph-CH=CH-CO-, which is more polar because of the incorporation of the hydroxyl. Conversely, several analogues of 3 reported in the literature possess low nanomolar affinities against MC1R with varying selectivities. Thus, it seems reasonable to conclude that the loss in affinity experienced by 3 results from the incorporation of the alkene rather than the increased polarity that arises from the addition of the hydroxyl group. While the exact reasons behind the affinity of 3, or lack thereof, remain unclear, it is plausible that incorporation of the alkene in this ligand causes the peptide to adopt too rigid a structure, thereby reducing its ability to conform to the receptor binding pocket.

Ligands 5–8 are about twice as large and display binding affinities 1–2 orders of magnitude higher with hMC1R compared to those described above. Ligands 6 and 8 were synthesized as analogues of ligands 5 and 7, respectively, to be used for potential attachment to nanoparticles. The similarity in binding affinities of 5 versus 6 and 7 versus 8 further demonstrates that the C-terminal end of these peptides is a suitable location for the placement of an attachment of a scaffold, as it does not seem to impact the binding ability of the ligand.

A targeted, stabilized triblock polymer micelle was prepared by combining 10% 4-targeted polymer with 90% untargeted polymer (Scheme 2). As a control, competitive binding assays were performed with targeted and untargeted polymer, as well as untargeted stabilized micelles. For consistency, all binding assays were normalized to the concentration of the ligand. As previously stated, the 4-targeted micelle exhibits an increased binding avidity to the hMC1R receptor compared to the targeted polymer and a slightly weaker avidity than the native ligand. The increase in binding avidity for the targeted micelles compared to the targeted polymer is noteworthy in that (1) it demonstrates the *in vitro* stability of this micelle system and (2) it indicates that the binding avidity of the 4-targeted micelles may be benefiting from multivalent interactions. Additionally, the 4-targeted polymer and 4-targeted micelle demonstrated no measurable interactions with either MC4R or MC5R, thereby indicating that the targeted micelle is itself more specific than the ligand alone.

The decrease in binding affinity of the targeted polymer relative to the native ligand can be explained by the conjugation of a large, flexible PEG group to a relatively small ligand. In addition to adding to the entropy of the ligand system through increased flexibility and size, PEG chains are known to have at least moderate interaction with nonpolar hydrophobic groups.¹⁸ Consequently, it is possible that the PEG moiety on the end of the triblock polymer is weakly interacting with the hydrophobic amino acids of the targeting group, thereby decreasing its affinity for MC1R.

However tempting it may be to ascribe the modest increase in avidity of the targeted micelles relative to targeted polymer solely to multivalent binding, such interactions are of high complexity and can be attributed to the sum of multiple factors. These variables include the enthalpies of individual binding events, the entropic consequences experienced by the micelle polymer upon binding, the topography of ligand positioning on the micelle, the statistical proximity effect, and differences in rates of cellular uptake.

Improved avidity through multivalent interactions is more readily observed with ligands with relatively low affinity.¹⁹ In the case of 4, it is possible that multivalent interactions would not greatly enhance binding avidity given the high affinity of the targeting ligand for binding the MC1R receptor. Also, assembled polymer micelles have decreased entropy relative to free polymer, via the hydrophobic effect, thereby potentially leading to slower ligand–receptor off rates.²⁰ Additionally, ligand proximity and steric repulsions between ligands and polymer chains are known to be important factors that influence the degree, if any, to which multivalency is experienced in micellar systems.^{19b}

The targeted micelles herein are calculated to have approximately 10 targeting groups each. This may seem to be a large number; however, since these stabilized micelles are relatively inflexible, ligands binding to the surface of a cell

positioned on one side of the micelle could prohibit binding of ligands positioned on the opposite side. Since total ligand concentration was considered when calculating avidities, having a large fraction of ligands inaccessible to receptor at any given time would effectively reduce the calculated avidity. However, even if multivalent binding is completely inhibited in this manner, increased binding avidity can be still observed because of the statistical effect where proximal ligands can readily bind following release of an existing interaction.²¹

Lastly, the rate of cellular uptake is likely different for the targeted micelle relative to the targeted polymer or free ligand. Constructs with faster rates of uptake can be calculated to have higher avidities due to the loss of “off-rate” once internalized. The sum of these variables and others not mentioned may contribute to the observed binding avidity of these supra-molecular systems.

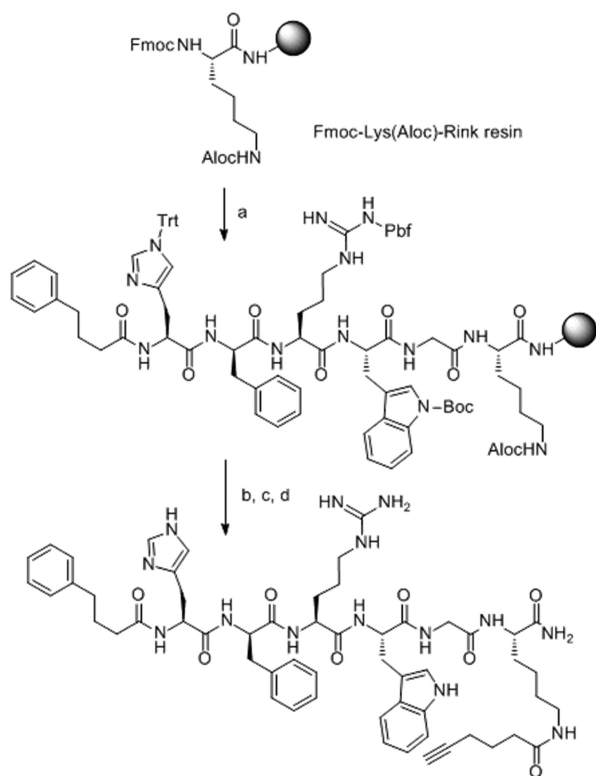
CONCLUSION

An hMC1R ligand was modified for attachment to a stabilized triblock polymer micelle. Functionalization and subsequent attachment of the ligand to a ~100 nm polymer micelle resulted in a slight decrease in affinity, but increase in specificity, to MC1R. Presumably, this decrease results from the thermodynamic hurdles encountered in appending a small peptide to a large nanoparticle, as well as an inherent handicap in the assay design. As mentioned in the Introduction, three hurdles must be overcome in the design of an effective targeted nanoparticle delivery system: (1) it must be ensured that there is no loss of ligand affinity resulting from the attachment of a small peptide to a large nanoparticle, or any such loss in affinity must be compensated by multivalent binding interactions; (2) nanoparticles must be inherently stable; (3) nanoparticles must be sufficiently small to escape the vasculature and enter the tumor. We believe the current publication addresses the first two of these concerns. We have shown that our ligand remains selective after attachment and the increased binding affinity observed between the 4-targeted polymer and 4-targeted stabilized micelle has demonstrated the *in vitro* stability of the system. On the basis of our DLS data, we are confident that our micelles are of sufficient size to escape the vasculature, and *in vivo* studies to evaluate the selectivity and stability of this targeted micellar system in mice are currently underway.

EXPERIMENTAL SECTION

Ligand Synthesis. *N*- α -Fmoc-protected amino acids, HBTU, HCTU, HOCT, and HOBt were purchased from Anaspec (San Jose, CA) or Novabiochem (San Diego, CA). Rink amide Tentagel S and R resins were acquired from Rapp Polymere (Tubingen, Germany). Rink amide 1% DVB PS resin was acquired from Novabiochem (San Diego, CA). For the *N*- α -Fmoc-protected amino acids, the following side chain protecting groups were used: Arg(*N*^ε-Pbf); Asp(*O*-*t*Bu); His(*N*^{im}-Trt); Trp(*N*¹-Boc); Tyr(*t*Bu); Lys(*N*^ε-Alloc). Reagent grade solvents, reagents, and acetonitrile (ACN) for HPLC were acquired from VWR (West Chester, PA) or Aldrich-Sigma (Milwaukee, WI) and were used without further purification unless otherwise noted. *N*-Terminal heterocyclic acids, NMI, and scavengers were obtained from Sigma-Aldrich or TCI. The solid-phase synthesis was performed in fritted syringes using a Domino manual synthesizer obtained from Torviq (Niles, MI). The C-18 Sep-Pak RC cartridges for solid phase extraction were purchased from Waters (Milford, MA).

Ligands 1–8 were prepared as previously published by solid-phase synthesis as summarized in Scheme 1 on Rink amide Tentagel resin (0.23 mmol/g) using a Fmoc/*t*Bu synthetic strategy and standard

Scheme 1. Synthetic Route for Compounds 1–8^a

^a(a) (i) Fmoc-AA-OH (3 equiv), HOCT or HOBt (3 equiv), and DIC (3 equiv) in DMF/DCM (10 mL/1g of resin) for amino acid couplings; (ii) piperidine/DMF (1:10, 2 + 20 min); (iii) 4-phenylbutyric acid (6 equiv), and DIC (3 equiv) in DMF/DCM; (b) (i) Pd(0) tetrakis(triphenylphosphine) (0.01 equiv), *N,N'*-dimethylbarbituric acid (5 equiv) in degassed DCM (2 × 30 min); (ii) 5-hexynoic acid (5 equiv) and DIC (3 equiv) in DMF/DCM for compound 1; *S*-Trt-3-propanoic acid (5 equiv) and DIC (3 equiv) in DMF/DCM for compound 2; (c) (i) TFA-scavenger cocktail (91% TFA, 3% water, 3% thioanisole, 3% ethanedithiol); (ii) ether extraction; (d) purification.

activations.^{15,22} After final deprotection of the Fmoc group, the resin was coupled with HOBt ester of 4-phenylbutyric acid (compounds 1 and 4), acetylated with acetic anhydride/pyridine (compound 2), or left unreacted as a free amino group (compounds 5–8). 4-Hydroxycinnamoyl-His-DPhe-Arg-Trp-NH-resin was treated with 50% piperidine in DMF to remove 4-hydroxycinnamoyl oligomers. The ligands were cleaved off the resins with TFA-scavenger cocktail (91% TFA, 3% water, 3% thioanisole, 3% ethanedithiol), extracted with cold diethyl ether, then dissolved in 1.0 M aqueous acetic acid. The crude ligands were purified by SEC and HPLC. All final compounds were >95% pure by HPLC analysis. The pure compounds were dissolved in deionized water or DMSO at approximately 1.0 mM, and concentration was determined by Trp HPLC measurement.²³ Ligand purification methods, mass spectra, and HPLC characterization data are provided in Supporting Information

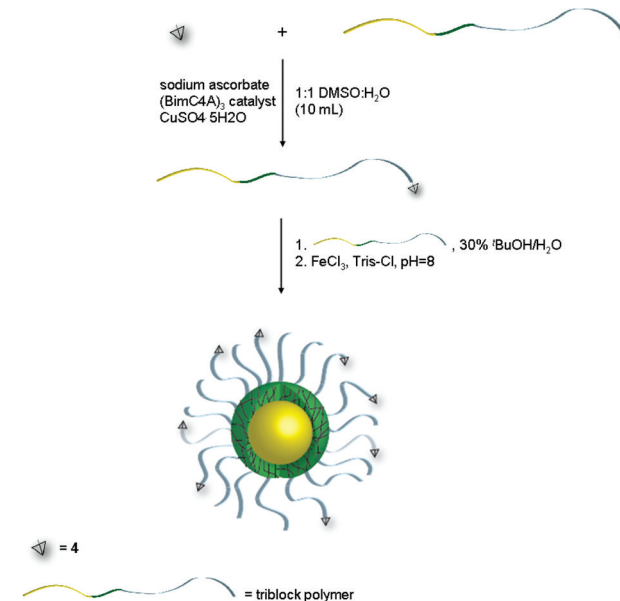
Cell Culture. HCT116 cells overexpressing hMC1R, and HEK293 cells overexpressing hMC4R^{13b} or hMC5R were used in all studies. The parental human colorectal carcinoma cell line HCT116 (American Type Culture Collection, CCL 247) was also used. Cells were maintained under standard conditions (37 °C and 5% CO₂) and were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS and 5% penicillin/streptomycin. For HCT116/hMC1R cells, Geneticin (G418S, 0.4 mg/mL) was added to the medium to ensure proper selection.

Europium Binding Assays. Competitive binding assays were performed using HCT116/hMC1R cells and HEK293/hMC4R or

hMC5R as previously described, with slight modifications.^{13b} HCT116/hMC1R cells were plated in black PerkinElmer 96-well plates, and HEK293/hMC4R and HEK293/hMC5R cells were plated on SigmaScreen poly-D-lysine coated plates (Sigma-Aldrich), all at a density of 10000–30,000 cells/well. Poly-D-lysine coated plates contain a PDL polymer coating that creates a uniform positive charge at the surface of the plastic, thereby facilitating cell attachment, growth, and differentiation. Both PerkinElmer and SigmaScreen plates were evaluated for nonspecific binding. Cells were grown in the 96-well plates for 2–3 days. On the day of the experiment, the medium was aspirated and 50 μL of nonlabeled competing ligand was added to each well in a series of decreasing concentrations (ranging from ~1 μM to 0.1 nM), followed by 50 μL of Eu-NDP- α -MSH at 10 nM. Both labeled and nonlabeled ligands were diluted in binding medium (DMEM, 1 mM 1,10-phenanthroline, 200 mg/L bacitracin, 0.5 mg/L leupeptin, 0.3% BSA). In the case of the triblock polymer micelle solutions, micelles were allowed to equilibrate in solution for 30 min prior to cell addition. Cells were incubated with labeled and nonlabeled ligands for 1 h at 37 °C. Following incubation, cells were washed three times with wash buffer (50 mM Tris-HCl, 0.2% BSA, 30 mM NaCl), and 100 μL of enhancement solution (PerkinElmer) was added to each well. Cells were incubated for an additional 30 min at 37 °C prior to reading. The plates were read on a PerkinElmer VICTORx4 2030 multilabel reader using the standard Eu time-resolved fluorescence (TRF) measurement (340 nm excitation, 400 μs delay, and emission collection for 400 μs at 615 nm). Competition curves were analyzed with GraphPad Prism software using the sigmoidal dose–response (variable slope) classical equation for nonlinear regression analysis.

Synthesis of Targeted Triblock Polymers. Triblock polymer, azido-poly(ethylene glycol)-*block*-poly(aspartic acid)-*block*-poly(leucine-*co*-tyrosine), was obtained from Intezyme Inc. (Tampa, FL). Alkyne functionalized ligand 4 was conjugated to the terminal azide of the polymer by copper assisted click chemistry (CuAAC)²⁴ (Scheme

Scheme 2. Synthesis of Targeted, Stabilized Micelle System



2) using the following method. To a solution of 1:1 DMSO/H₂O (10 mL) was added 4 (16 mmol, 1.2 equiv), triblock polymer (13.3 mmol, 1 equiv), sodium ascorbate (334.15 mmol, 25 equiv), (BimC₄A)₃ catalyst²⁵ (13.42 mmol, 0.2 equiv), and CuSO₄·5H₂O (13.3 mmol, 1 equiv). The solution was heated to 50 °C and stirred for 2 days. The mixture was then cooled and placed in a 3500 MW dialysis bag (Spectra Por) and dialyzed against EDTA/H₂O (×3) and H₂O (×3). Following purification by dialysis, the solution was lyophilized.

Successful click coupling was verified through visualization of the triazole-H in ^1H NMR (8.02 ppm).

Micelle Formulation. Triblock polymers were dissolved at 20 mg/mL in 30% *tert*-butanol/ H_2O at room temperature, stirred for 4 h, and then lyophilized (Scheme 2).²⁶ Micelles were stabilized with an Fe(III) cross-linking (Scheme 2) by dissolving the micelle (20 mg/mL) in FeCl_3 (1.35 mg/mL) in Tris buffer, adjusting to pH 8, and stirring for 12 h. For the targeted micelle system, 10% targeted polymer and 90% untargeted polymer were used in the formulation mixture. Micelle size was determined by dynamic light scattering (DLS, Wyatt Technology, DynaPro), and surface charge was determined by ζ measurement (Malvern, Zetasizer).

■ ASSOCIATED CONTENT

● Supporting Information

Complete information on general methods and synthesis, solid phase synthesis QC and purification, and mass spectrometry. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*For D.L.M.: phone, 813-745-8948; fax, 813-745-8357; e-mail, david.morse@moffitt.org. For J.V.: phone, 520-626-4179; fax, 520-626-4824; e-mail, vagner@email.arizona.edu.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

■ ACKNOWLEDGMENTS

The authors acknowledge Intezyne Inc. for support and scientific contributions. Research was funded by the Bankhead Coley Melanoma Pre-SPORE Program (Grant 02-15066-10-03 to D.L.M.), NIH/NCI (Grant R01 CA 097360 to R.J.G. and D.L.M.), and Intezyne Inc. (Award 84-16301-01-01 to R.J.G.) for a portion of N.M.B.'s salary and polymer materials.

■ ABBREVIATIONS USED

Aloc, allyloxycarbonyl; $(\text{BimC}_4\text{A})_3$, potassium 5,5',5''-(2,2',2''-(nitrilotris(methylene))tris(1*H*-benzimidazole-2,1-diyl))-tripentanoate; Boc, *tert*-butyloxycarbonyl; ^tBu , *tert*-butyl; DMSO, dimethylsulfoxide; DVB, divinylbenzene; Fmoc, (9*H*-fluoren-9-ylmethoxy)carbonyl; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBT, *N*-hydroxybenzotriazole; HOCT, 6-chloro-1*H*-hydroxybenzotriazole; NMI, *N*-methylimidazole; Pbf, 2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl; PS, polystyrene; RP-HPLC, reverse-phase high performance liquid chromatography; TFA, trifluoroacetic acid; Trt, triphenylmethyl (trityl)

■ ADDITIONAL NOTE

Abbreviations used for amino acids and designation of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1972**, *247*, 977–983.

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