Chapter 3B

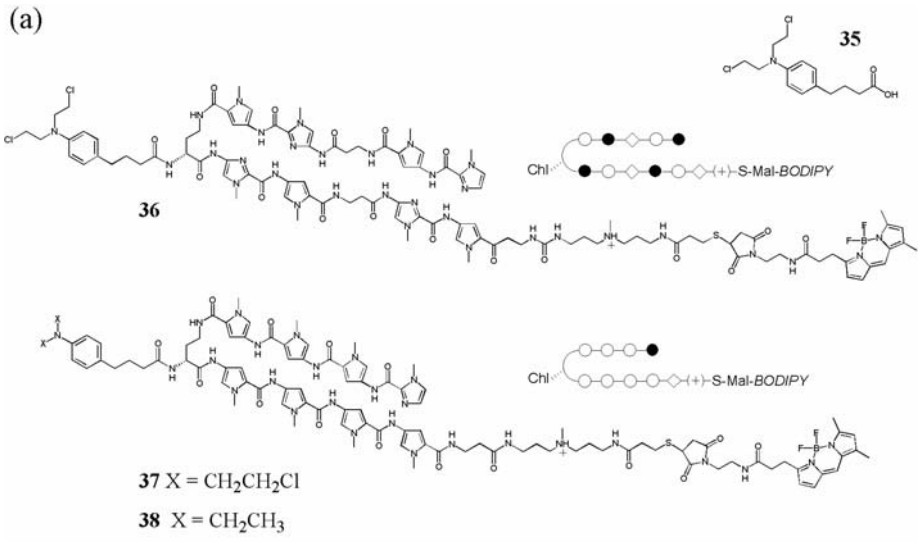
Polyamide-Small Molecule Conjugates for Cellular Uptake Studies

Introduction

Continuing experiments aimed at creating polyamides or polyamide conjugates that cross the outer membranes of living cells and transit to the nucleus resulted in the creation of several small molecule-polyamide conjugates. The first series of such compounds studied incorporated the DNA-alkylating agent chlorambucil (CHL, **35**). The laboratories of both Terry Beerman and Joel Gottesfeld have observed effects such as toxicity and DNA-alkylation in a variety of cell lines with polyamide-CHL conjugates.[29] As they were producing an effect in living cells, it was interesting to test Bodipy-polyamide-CHL conjugates for cellular localization. Compounds **36**-**38** were synthesized and tested in a wide panel of cell lines, localizing to the cytoplasm of all cell lines save NB4, CEM, and human primary CD4+ T-cells (Figure 3.15). These cell lines exhibited nuclear staining upon treatment with **36** and **37**, without accompanying toxicity at the concentration studied (5 μM).

The successful staining of NB4 nuclei suggested that further attempts to optimize the uptake characteristics of Bodipy-polyamide-CHL conjugates might meet with some success. Many small molecules serving as nutrients or signal transduction messengers are taken up into cells by specific transmembrane protein receptors. It has been shown that the cellular uptake of several types of biopolymers, including DNA, peptide nucleic acids, and proteins, can be increased when attached to receptor-specific small molecules.[30-32] The folate receptor is a frequent target of these compounds, since its process of uptake by receptor-mediated endocytosis is well understood and the receptor is

(a)



(b)

Conjugate	PC3	CEM	NB4	SKBR-3	MCF-7	MEL	293	LnCap	Kc	Sf9
36	Cyt	Nuclear	Nuclear	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt
37	Cyt	Nuclear	Nuclear	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt
38	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt	Cyt

Figure 3.15 Bodipy-polyamide-chlorambucil conjugates for cell uptake studies. (a) Chemical structures and ball-and-stick models of chlorambucil, **35**, and Bodipy-polyamide-CHL conjugates **36**[29] and **37**-**38**[25]. (b) The designation 'nuclear' indicates observation of fluorescence in the interior of the nucleus. The designation 'cyt' indicates cellular, non-nuclear fluorescence.

overexpressed in several cancers.[33] In order to exploit cell receptors as possible paths of cellular influx, Bodipy-polyamide conjugates with folic acid and cholic acid were synthesized and tested in cell uptake assays (Figure 3.16). These compounds were ineffective at increasing cellular uptake in the standard panel of cell lines. However, other small-molecule conjugates might have increased uptake if the operative receptor-targeting moiety is attached to the polyamide at the optimal linkage site, using an optimal linking domain.

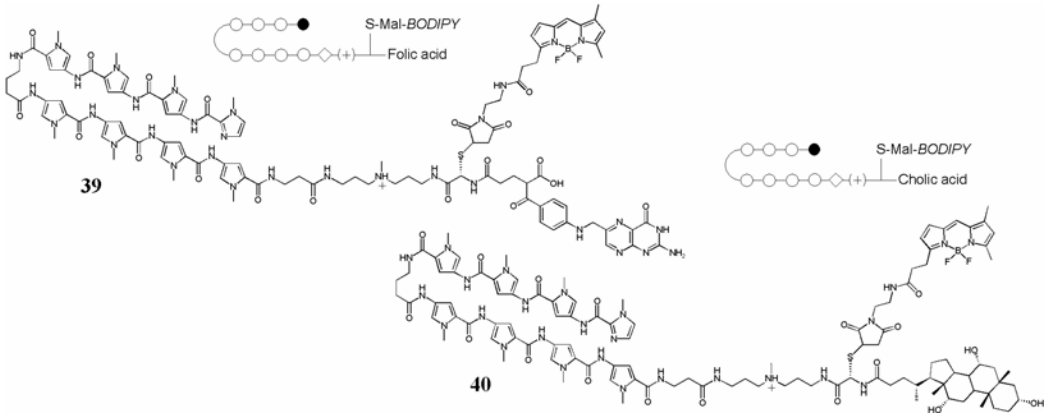
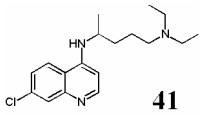


Figure 3.16 Bodipy-polyamide-small molecule conjugates for cell uptake studies.[25]

Most fluorescent conjugates studied show localization in the cytoplasm of living cells in a punctate pattern. This suggests that polyamides and polyamide conjugates are, by and large, able to cross the cellular membrane, but are then tied up in cytoplasmic vesicles such as endosomes or lysosomes. Another possible method to increase the uptake of polyamides and polyamide conjugates in living cells is co-treatment of the cells with drugs. A known endosomal disruption agent, chloroquine (**41**), was studied as one possible formulation agent for polyamide-Bodipy conjugate **2**.[34] At 10 μM added chloroquine, **2** was found to stain the nuclei of PC3 and MEL cells. At higher chloroquine concentrations (~100 μM) the cells showed toxicity, illustrating that any added agent must be both potent and specific in its effect, in order that the triggering of stress mechanisms or apoptosis be avoided.

The success of chloroquine in increasing the uptake of a polyamide conjugate suggests that other drugs, such as those known to inhibit the multidrug response (MDR) might be effective formulating agents.[35]



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Results

Polyamide-chlorambucil conjugates

Two Bodipy-polyamide-CHL conjugates were designed as alternate morphologies of **36**-**38** to hopefully increase their cellular uptake and nuclear staining properties beyond merely NB4, CEM, and CD4+ T-cells. Compounds **42** and **43** were synthesized as in Figure 3.17. The necessity of having three differentially-protected amine moieties on **43** led to the use of pyrrole monomer **46**, possessing an *N*-propylamine masked as an azide. After addition of the CHL moiety, the azide was cleanly and specifically converted to an amine via the Staudinger reaction, leaving the CHL moiety intact. This strategy of amine protection/deprotection may prove useful in the future in the synthesis of complex polyamide conjugates. Unfortunately, upon exposure to the standard panel of cell lines, both **42** and **43** were found to localize to the cytoplasm in all cases.

Polyamide-dihydrotestosterone conjugate

Dihydrotestosterone (DHT), a steroid hormone, has been used to target prostate cancer cells for uptake.[30] Based on this, and pursuant to an ongoing collaboration with ZhengxinWang's lab at the University of Texas Southwestern Medical Center (see chapter 5), compound **50** was synthesized (Figure 3.18). This fluorescent compound incorporates a hydrophilic polyethylene glycol (PEG) linking domain between the polyamide and steroid moieties. It was hoped that the increased hydrophilicity of the compound, due to the linking domain, and the receptor-targeting ability of the DHT domain would afford increased uptake in prostate cancer cell lines PC3 and LNCaP. Indeed, a comparison of the uptake of **2** with **50** shows marked increase in cytoplasmic

staining in PC3 and LNCaP cells. However, no nuclear staining is detected, suggesting that the novel domains do act to increase the transit of the polyamide across the cellular membrane, but are unable to overcome the sequestration of compound in cytoplasmic vesicles.

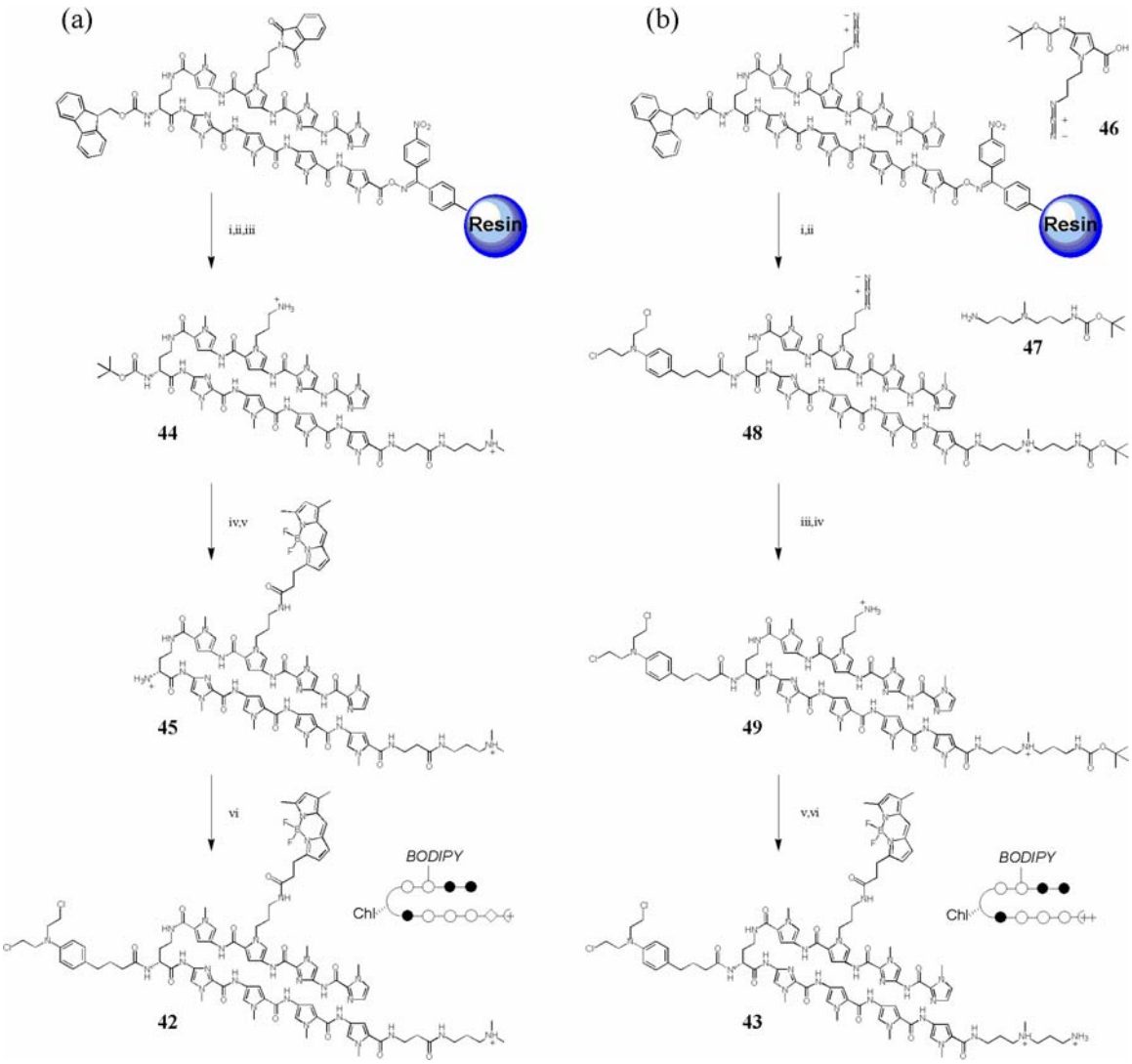

Figure 3.17 Synthesis of CHL conjugates. (a): (i) 20% piperidine/DMF (v/v), 30 min at room temperature. (ii) Boc_2O, DIEA, DMF. (iii) 3-dimethylaminopropylamine, 6 hrs at 37ºC. (iv) Bodipy-FL, HOBt, DCC, DIEA, DMF. (v) 20% TFA in CH_2Cl_2 (v/v), 30 min at room temperature. (vi) **35**, HOBt, DCC, DIEA, DMF. (b): (i) **47**, 18 hrs at 37ºC. (ii) **35**, HOBt, DCC, DIEA, DMF. (iii) THF, PPh_3, 3 hrs at room temperature. (iv) $NaOH_{(aq)}$ 1 eq, 2 hrs at 70ºC. (v) Bodipy-FL, HOBt, DCC, DIEA, DMF. (vi) $BF_3{\cdot}(Et_2O)_2$, CH_2Cl_2, 30 min at room temperature.

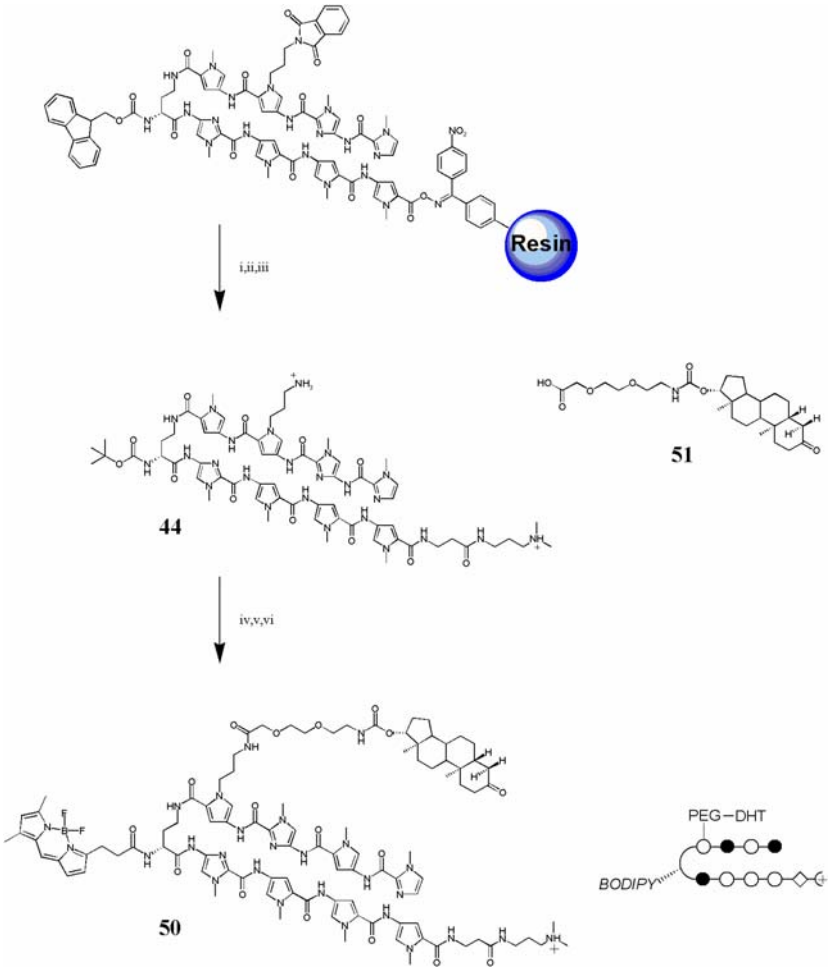

Figure 3.18 Synthesis of DHT conjugate. (i) 20% piperidine/DMF (v/v), 30 min at room temperature. (ii) Boc_2O, DIEA, DMF. (iii) 3-dimethylaminopropylamine, 6 hrs at 37ºC. (iv) **51** (for synthesis, see chapter 5, compound **13**), HOBt, DCC, DIEA, DMF. (v) 20% TFA in CH_2Cl_2 (v/v), 30 min at room temperature. (vi) Bodipy-FL, HOBt, DCC, DIEA, DMF.

Experimental Section

Synthesis of Polyamide Conjugates 1-18, 36-40

These conjugates were synthesized by P. S. Arora and J. M. Belitsky.[25,37]

Synthesis of 26[38]

Maleimide (1 g, 10 mmol), methyl chloroformate (0.8 mL, 10 mmol), and *N*-methylmorpholine (1.13 mL, 10 mmol) were dissolved in EtOAc (50 mL) at 0ºC and stirred for 1 hr. The resulting white precipitate was removed by filtration and the filtrate concentrated to obtain a pink solid. Boc- (*D*) Lys-OH (0.9 g, 3.5 mmol) was dissolved in sat. $NaHCO_3$ solution (35 mL) at 0ºC, to which was added the pink solid and the resulting suspension was stirred for 4 hrs, with slow warming to room temperature. The solution was extracted with $CHCl_3$ (2 x 150 mL), and the aqueous layer was acidified to pH~3 with concentrated H_2SO_4. The solution was washed with $CHCl_3$ (3 x 100 mL). The organic fractions were combined, dried with $MgSO_4$, and concentrated to yield a translucent yellow oil.

Synthesis of Peptide-Polyamide-Bodipy Conjugates 19-24

The synthesis of the 1-[3-(*tert*-butoxycarbonyl)amino]propyl-imidazole-2-carboxylic acid monomer utilized in the synthesis of **19-24** has been recorded.[29] Polyamide was synthesized on Kaiser oxime resin by standard solid phase methods as previously reported and liberated upon treatment with methylamine to provide **25**.[27,28] **26** (230 μmol) was dissolved in 300 μL DMF, to which was added DCC (230 μmol, 100 μL DMF) and HOBt (230 μmol, 100 μL DMF), and the resulting solution allowed to react at

37ºC for 30 min. This solution was added to **25** (70 μmol), dissolved in DMF (500 μL) and DIEA (40 μL, 230 μmol), and allowed to react at room temperature for 3 hrs, until complete as monitored by analytical HPLC. The crude product was dissolved into ~10 mL CH_2Cl_2, to which was added ~10 mL TFA and the resulting solution stirred at room temperature for 30 min. The solution was concentrated, purified by C_{18} reverse-phase preparatory HPLC, and lyophilized to yield a white powder. To 10 μmol of this polyamide dissolved in DMF (185 μL), was added first a solution of Bodipy-FL (20 μmol), DCC (20 μmol), and HOBt (20 μmol) dissolved in 300 μL DMF allowed to react at room temperature for 30 min, then DIEA (3.5 μL, 20 μmol), and the resulting solution was allowed to react at room temperature for 3 hrs. To 100 μL of this solution was added a solution of the appropriate peptide bearing an N-terminal cysteine residue (5 μmol) and TCEP (7.5 μmol) dissolved in 6M Gn·HCl (100 μL, pH = 7.3). The resulting solution was allowed to react at room temperature for 1 hr, and then purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **19**-**24** as orange powders. Characterization: **19** [(*D*) C-TAT*-PA-Bodipy], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2913.9, obsd 2914.4; **20** [(*L*) C-NLS-PA-Bodipy], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2594.2, obsd 2594.4; **21** [(*L*) C-TAT-PA-Bodipy], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 3270.0, obsd 3270.6; **22** [(*D*) C-R_7-PA-Bodipy], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2821.4, obsd 2821.7; **23** [(*D*) C-R_9-PA-Bodipy], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 3132.5, obsd 3132.9; **24** [(*D*) C-$(RAhx)_6$R-PA-Bodipy], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 3500.3, obsd 3500.4.

Synthesis of Polyamide-Peptide Conjugates 27-32

25 (35 μmol) was dissolved in 600 μL DMF and treated with DIEA (59 μL, 350 μmol) and a solution of 3-maleimidopropionic acid (0.028 g, 175 μmol), DCC (0.035 g, 165 μmol) and HOBt (0.024 g, 175 μmol) reacted for 30 min at room temperature in 200 μL DMF. The resulting solution was allowed to react at room temperature for 3 hrs then purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield a white powder. The maleimido-polyamide (2 μmol) was dissolved in 160 μL DMF, to which was added a solution of TCEP (4 μmol) and peptide (10 μmol) in 200 μL Gn·HCl (6 M, pH = 7.3). The resulting solution was allowed to react at room temperature for 1 hr, and then purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **27-32** as white powders. Characterization: **27** [(*L*) C-NLS-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2261.4, obsd 2261.1; **28** [(*L*) C-TAT-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2936.1, obsd 2935.5; **29** [(*D*) C-TAT*-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2582.1, obsd 2581.8; **30** [(*D*) C-R_7-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2489.3, obsd 2489.2; **31** [(*D*) C-R_9-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2801.4, obsd 2801.1; **32** [(*D*) C-$(RAhx)_6$R-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 3168.0, obsd 3166.8.

Synthesis of Polyamide-Disulfide-Peptide Conjugates 33-34

Polyamide was synthesized on Kaiser oxime resin employing the 1-[3-(fluorenylmethyloxycarbonyl)amino]propyl-4-(*tert*-butoxycarbonyl)amino-pyrrole-2-carboxylic acid monomer, whose synthesis has been detailed elsewhere.[29] The Fmoc protecting group was removed upon treatment of the resin with 20% (v/v)

piperidine/DMF, and replaced with the *t*-Boc protecting group by treatment of the resin with Boc_2O (0.5 g, 2.3 mmol) and DIEA (400 μL, 2.3 mmol) in 4 mL NMP. The polyamide was liberated from resin by treatment with 3,3'-diamino-*N*-methyldipropylamine for 10 hrs at room temperature and purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **35** as a white powder.

S-trityl-3-mercaptopropionic acid (0.026 g, 75 μmol), DCC (75 μmol), and HOBt (75 μmol) were dissolved in 225 μL DMF and allowed to activate for 3 hrs at room temperature. The resulting solution was centrifuged to remove precipitated DCU and added to a solution of **35** (11 μmol), dissolved in 300 μL DMF, and DIEA (15 μL, 90 μmol) was added. This was allowed to react for 18 hrs at room temperature. Dp (8 μL) was added (to quench any remaining *S*-trityl-3-mercaptopropionic acid –OBt ester), then the crude product was precipitated by addition of Et_2O and treated with 50% (v/v) TFA in CH_2Cl_2 for 1 hr at room temperature. The resulting solution was purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **36** as a white powder.

A solution of Bodipy-FL (17 μmol), DCC (17 μmol), and HOBt (17 μmol) was prepared in 200 μL DMF and allowed to activate for 2 hrs at room temperature. This was added to a solution of **36** (9 μmol) in DMF (300 μL) and DIEA (10 μL, 60 μmol) and allowed to react at room temperature for 1 hr. This solution was purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **37** and the thioester of **37** and Bodipy (1:1) as orange powders. The thioester was treated with 10 μL Dp to cleave the Bodipy thioester and purified to yield **37**.

Each peptide (2 μmol) was dissolved in 75 μL DMF. 2-Aldrithiol (0.5 μmol) was added and the solutions allowed to react for 1 min. A solution of **37** in 75 μL DMF was

then added to each peptide solution and allowed to react for 4 hrs. They were then purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **33** and **34** as orange powders. Characterization: **33** [(*D*) C-TAT*-SS-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2905.4, obsd 2907.8; **34** [(*D*) C-R_9-PA], MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 3124.7, obsd 3126.3.

Synthesis of Bodipy-Polyamide-Chlorambucil Conjugate (42)

Polyamide was synthesized on Boc-β-Ala-PAM resin employing the 1-[3-phthalimido)amino]propyl-4-(*tert*-butoxycarbonyl)amino-pyrrole-2-carboxylic acid monomer, whose synthesis has been reported elsewhere.[39] The Fmoc protecting group on the DABA turn was removed upon treatment of the resin with 20% (v/v) piperidine/DMF, and replaced with the *t*-Boc protecting group by treatment of the resin with Boc_2O (0.64 g, 3.0 mmol) and DIEA (200 μL, 1.7 mmol) in 3 mL DMF. The polyamide was liberated from resin by treatment with Dp for 18 hrs at 37ºC and purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **44** as a white powder.

A solution of Bodipy-FL (22 μmol), DCC (22 μmol), and HOBt (22 μmol) was prepared in 265 μL DMF and allowed to activate for 1 hr at room temperature. To this was added a solution of **44** (11 μmol) and DIEA (6 μL, 34 μmol), and the resulting solution was allowed to react at room temperature for 10 hrs. The crude product was precipitated by addition of Et_2O and treated with 20% (v/v) TFA in CH_2Cl_2 for 30 min at room temperature. The resulting solution was purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield **45** as an orange powder.

A solution of chlorambucil (0.009 g, 30 μmol), DCC (30 μmol), and HOBt (30 μmol) was prepared in 200 μL DMF and allowed to activate for 1 hr at room temperature. To the resulting solution was added a solution of **45** (3 μmol) in 350 μL DMF and DIEA (2 μL, 10 μmol), which was allowed to react for 3 hrs at room temperature, then purified by C_{18} reverse-phase preparatory HPLC and lyophilized to provide **42** as an orange powder. Characterization: ESI peaks of $[M+H]^+$ (monoisotopic mass ^{35}Cl) calcd 1840.9, obsd 1841.1; $[M+H]^+$ (isotopic mix of one ^{35}Cl and one ^{36}Cl) calcd 1841.9, obsd 1842.4; $[M+H]^+$ (monoisotopic mass ^{36}Cl) calcd 1842.9, obsd 1843.3.

Synthesis of Bodipy-Polyamide-Chlorambucil Conjugate (43)

Polyamide was synthesized on Kaiser oxime resin using azide pyrrole monomer **46** and liberated by cleavage with **47** (~1g, ~4 mmol) in 1 mL NMP. This solution was purified by C_{18} reverse-phase preparatory HPLC and lyophilized to provide polyamide as a white powder. The polyamide (8.8 μmol) was treated with an activated solution of chlorambucil (45 μmol), DCC (45 μmol), and HOBt (45μmol), as well as with DIEA (15 μL, 90 μmol). The resulting solution was allowed to react at room temperature for 3 hrs, then the crude product **48** was precipitated by addition of Et_2O.

Solutions of **48** (4.5 μmol) and triphenylphosphine (4.5 μmol) were prepared in anhydrous THF, then mixed and allowed to react for 3 hrs at room temperature. To this was then added aqueous NaOH (4.5 μmol) and the resulting solution heated at 70ºC for 2 hrs, until complete by analytical HPLC. The crude product **49** was precipitated by addition of Et_2O, then treated with an activated solution of Bodipy-FL (11 μmol), DCC (11 μmol), and HOBt (11 μmol), and DIEA (15 μL, 11 μmol). The crude product was

precipitated by addition of Et_2O, then resuspended in 100 μL anhydrous CH_2Cl_2, to which was added 10 μL $BF_3{\cdot}(Et_2O)_2$ and the solution allowed to react at room temperature for 30 min. The crude conjugate was precipitated upon addition of Et_2O and purified by C_{18} reverse-phase preparatory HPLC and lyophilized to provide **43** as an orange powder. Characterization: ESI peaks of $[M+H]^+$ (monoisotopic mass ^{35}Cl) calcd 1812.9, obsd 1812.5; $[M+H]^+$ (isotopic mix of one ^{35}Cl and one ^{36}Cl) calcd 1813.9, obsd 1813.5; $[M+H]^+$ (monoisotopic mass ^{36}Cl) calcd 1814.9, obsd 1814.6.

Synthesis of 1-[3-(azido)propyl]-4-(*tert*-butoxycarbonyl)amino-pyrrole 2-carboxylic acid (46)

Ethyl-4-nitropyrrole-2-carboxylate (2.5g, 13 mmol) was dissolved in acetone, to which was added K_2CO_3, and the resulting suspension stirred vigorously at room temperature for 2 hrs. To this was added 3-iodopropanol (2.6 mL, 26 mmol), then the suspension was stirred vigorously under reflux for 2 hrs, 30 min. The slurry was cooled to room temperature, filtered, and concentrated to yield a yellow oil. To this was added 100 mL H_2O and the pH was adjusted to ~3 with 10% (v/v) H_2SO_4. The mixture was extracted with EtOAc (3 x 100 mL), dried ($MgSO_4$), concentrated, and purified on silica with 2:1 hexanes:EtOAc to yield a clear oil (3.6 g, 56% yield).

The ethyl-1-(3-hydroxyl)propyl-4-nitropyrrole-2-carboxylate (1.6 g, 6.8 mmol) was dissolved in 20 mL EtOAc, to which was added 10% (wt/wt) Pd/C (0.3 g, 0.28 mmol). The suspension was added to a Parr bomb, pressurized to 100 psi H_2, and stirred at room temperature for 2h. The mixture was filtered through celite to remove the Pd/C and concentrated. The resulting yellow oil was dissolved in 20 mL DMF, to which was

added DIEA (2.44 mL, 14 mmol) and Boc_2O (1.53 g, 7 mmol), and stirred at room temperature for 2 hrs. The solution was concentrated and purified on 75g silica with 1:1 hexanes:EtOAc to yield a clear oil (1.77g, 84% yield).

This oil (1g, 3.2 mmol) was dissolved in 10 mL CH_2Cl_2, to which was added triethylamine (0.67 mL, 4.9 mmol), and the resulting solution was cooled to 0ºC. The solution was then treated with mesyl chloride (0.33 mL, 4.2 mmol) and DMAP (0.16 g, 1.3 mmol), stirred at 0ºC for 15 min, then allowed to warm to room temperature while stirring for 1 hr. 40 mL CH_2Cl_2 was added and the solution was washed with 1M $KHSO_4$, H_2O, sat. $NaHCO_3$, H_2O, and brine (50 mL each), dried ($MgSO_4$), and concentrated.

The resulting oil was dissolved in DMF (10 mL), to which was added NaN_3 (1g, 16 mmol) and the resulting solution stirred at 55ºC under Ar for 1 hr 30 min. The solution was partitioned between 50 mL brine and 50 mL Et_2O. The aqueous layer was washed with an additional 30 mL Et_2O. The organic portions were combined and washed with 10% (v/v) citric acid, brine, 1M $NaHCO_3$, and brine (2 x 20 mL each), dried, and concentrated to a yellow oil. The oil was dissolved in 10 mL EtOH, to which was added 5 mL KOH, and stirred at 40ºC for 6 hrs. The solution was cooled to room temperature and acidified with 10% (v/v) H_2SO_4 to pH~3. The mixture was extracted with CH_2Cl_2 (3 x 40 mL), washed with brine, dried ($MgSO_4$), and concentrated to yield **46** as a pale yellow solid. ^1H NMR (300 mHz, $CDCl_3$): δ 7.25 (s, 1 H), 6.66 (s, 1 H), 4.40 (t, 2 H), 3.53 (t, 2 H), 1.96 (m), 1.50 (s, 9 H).

Synthesis of 3-(*tert*-butoxycarbonyl), 3'-diamino-*N*-methyldipropylamine (47)

3,3'-diamino-*N*-methyldipropylamine (32.2 mL, 200 mmol) was cooled to 0ºC. A solution of Boc_2O (4.4g, 20 mmol) was prepared in CH_2Cl_2 and added to cooled amine dropwise while stirring for 30 min. The solution was allowed to cool to room temperature while stirring for 1 hr 30 min. The solution was then extracted with 200 mL ¼ sat. $NaHCO_3$ (a volume of sat. $NaHCO_3$ diluted to 25% concentration). The aqueous solution was back-extracted with CH_2Cl_2 (4 x 50 mL), and the organic portions combined and washed with 50 mL sat. $NaHCO_3$ and 50 mL brine. The solution was dried with $MgSO_4$ and concentrated to yield a pearly-white oil, which was azeotroped with toluene (2.5 g, 55% yield). ^{1}H NMR (300 mHz, $CDCl_3$): δ 3.19 (br. q, 2 H), 2.81 (t, 2 H), 2.38 (m, 4 H), 1.65 (m, 4 H), 1.43 (s, 9 H).

Synthesis of Bodipy-Polyamide-DHT Conjugate (50)

Polyamide **44** was prepared as above. DHT-linker molecule **51** was prepared as detailed in Chapter 5. A solution of **51** (17 μmol), DCC (17 μmol), and HOBt (17 μmol) was prepared and allowed to react at room temperature for 30 min. To this was added a solution of **44** (3 μmol) and DIEA (1.6 μL, 10 μmol), and the resulting solution was allowed to react at room temperature for 2hrs. Dp (4 μL) was added to quench excess activated acid and the solution was concentrated with speedvac for 1 hr. The concentrated oil was treated with 1 mL 80% (v/v) TFA in CH_2Cl_2 for 2 hrs at room temperature, then was azeotroped with benzene 3x. The resulting oil was purified by C_{18} reverse-phase preparatory HPLC and lyophilized to yield DHT-polyamide as a white powder.

A solution of Bodipy-FL (3.6 μmol), DCC (3.6 μmol), and HOBt (3.6 μmol) was prepared and allowed to react for 30 min. To it was added the DHT-polyamide (1.8 μmol) and DIEA (0.5 μL, 5 μmol), and the resulting solution was allowed to react at 37ºC for 16 hrs. The solution was purified by C_8 reverse-phase preparatory HPLC and lyophilized to yield **50** as an orange powder. Characterization: MALDI-TOF $[M+H]^+$ (monoisotopic mass) calcd 2017.0, obsd 2017.8.

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