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# Efficient synthesis of N9-alkylguanines : & evaluation of a more highly conjugated analog of guanidinium-rich molecular transporters using a pH dependent fluorescence assay

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# EFFICIENT SYNTHESIS OF $N^9$-ALKYLGUANINES

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# EVALUATION OF A MORE HIGHLY CONJUGATED ANALOG OF GUANIDINIUM-RICH MOLECULAR TRANSPORTERS USING A pH DEPENDENT FLUORESCENCE ASSAY

By

David E. Olsen

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Submitted in partial fulfillment

of the requirements for

the Department of Chemistry

UNION COLLEGE

June, 2006

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**ABSTRACT**

OLSON, DAVID E. Efficient Synthesis of $N^9$-alkylguanines. Department of Chemistry, June 2006.

Synthesis of $N^9$-(2-chloroethyl)guanine from 2-amino-6-chloropurine was accomplished in three steps with an overall yield of 83%. All products were purified via crystallization and no chromatography was required. Selective crystallization of the desired $N^9$ isomer subsequent to the key alkylation step completely and efficiently removed the $N^7$ isomer. This compound was shown to be a versatile reagent in the synthesis of various $N^9$-substituted guanines.

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OLSON, DAVID E. Evaluation of a More Highly Conjugated Analog of Guanidinium-Rich Molecular Transporters Using a pH Dependent Fluorescence Assay. Department of Chemistry, June 2006.

The importance of the guanidino functional group in the cellular uptake of guanidinium-rich molecular transporters has been thoroughly demonstrated. Its ability to act as a bidentate hydrogen bond donor appears to play a key role in this process, but the effect of polarizability on cellular internalization has not been determined. We report the synthesis of a peptoid molecular transporter containing more highly conjugated guanine headgroups and its subsequent evaluation using a pH dependent fluorescence assay.

# Efficient Synthesis of N$^9$-alkylguanines

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## ABSTRACT

Synthesis of N$^9$-(2-chloroethyl)guanine from 2-amino-6-chloropurine was accomplished in three steps with an overall yield of 83%. All products were purified via crystallization and no chromatography was required. Selective crystallization of the desired N$^9$ isomer subsequent to the key alkylation step completely and efficiently removed the N$^7$ isomer. This compound was shown to be a versatile reagent in the synthesis of various N$^9$-substituted guanines.

*Keywords:* N$^9$-(2-chloroethyl)guanine; N-alkylation; anti-virals; N-substituted guanines.

The antiviral properties of N-substituted guanines are well known. [1-6] In particular, acyclovir and its analogs have received much attention.[7-11] Penciclovir and its pro-drug famciclovir have been shown to be effective anti-virals against varicella-zoster virus (VZV) and herpes simplex virus (HSV)[12], while entecavir has shown potent activity against hepatitis B virus (HBV).[13] Ganciclovir, another member of this class of

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compounds, has been used to treat cytomegalovirus (CMV) retinitis in immunocompromised patients.[14]

$N^9$-alkylsubstitution is a common structural motif in all of these guanine derivatives. However, selective $N^9$-alkylation has proven to be a synthetic challenge. Previous syntheses of $N^9$-substituted guanines have relied on the use of chromatography to separate the $N^7$ and $N^9$ regioisomers[15,16], but this method of separation is both burdensome and time-consuming. When 3-bromopropane-1,1,1-tricarboxylate was used as an alkylating agent, the $N^9$ isomer could be separated by crystallization, thus avoiding the use of chromatography.[17] Also, selective $N^9$-substitution has been achieved by using a blocking group at the $N^7$ position[18] as well as by the selective Pd-catalyzed allylation at the $N^9$ position.[19]

Herein, we report the facile synthesis of a functionalized $N^9$-alkyl guanine, namely $N^9$-(2-chloroethyl)guanine. Reaction of $N^9$-(2-chloroethyl)guanine with various nucleophiles may allow for the preparation of other $N^9$-substituted guanine derivatives with desirable medicinal properties.

The synthesis of $N^9$-(2-chloroethyl)guanine, **4**, is shown in Scheme 1. Conversion of 2-amino-6-chloropurine into the corresponding tetrabutylammonium salt, **1**, was accomplished in quantitative yield by the method of Bisacchi *et al.*[20] Next, N-alkylation was achieved by using an excess of 1-bromo-2-chloroethane, an inexpensive alkylating agent, under mild conditions. The reaction yielded a 5:1 ratio of regioisomers **2** and **3**,

the $N^7$ and $N^9$ substitution products, respectively. Recrystallization of the crude product from methylene chloride provided regioisomer **3** exclusively, thus eliminating the use of chromatography. Finally, hydrolysis of **3** in aqueous hydrochloric acid produced $N^9$-(2-chloroethyl)guanine, **4**, in nearly quantitative yield.

Many N-substituted guanines are easily accessible by the reaction of $N^9$-(2-chloroethyl)guanine with various nucleophiles. A few examples are reported in Scheme 2 and demonstrate the synthetic versatility of this compound. It should be noted that all nucleophilic substitution reactions involving $N^9$-(2-chloroethyl)guanine reported herein were not optimized.

$N^9$-(2-chloroethyl)guanine reacts smoothly with sodium iodide in DMF to provide the corresponding iodide. Not surprisingly, reaction of other nucleophiles with the iodide was considerably faster than with the chloride, so iodide catalysis was employed with subsequent nucleophiles. These reactions were run in DMSO-$d_6$, allowing them to be monitored by $^1$H-NMR. $^1$H-NMR analysis indicated that the first equivalent of either the amine, cyanide, thiolate, or carboxylate nucleophile resulted in the initial deprotonation of the lactam. Therefore, addition of a second equivalent was necessary to achieve the desired displacement.

All but one of the $S_N2$ reactions proceeded smoothly. Complications arose in the reaction with tetraethylammonium cyanide. Partial conversion to what is believed to be the desired substitution product was initially observed by $^1$H-NMR at 75 °C, but this

intermediate was degraded to products lacking the characteristic ethyl triplets observed in the $^{1}$H-NMR spectra of the other $N^9$-substituted products. We believe the desired product underwent elimination forming guanine and acrylonitrile. $^{1}$H-NMR analysis revealed succinonitrile, which would be expected from the addition of excess HCN to acrylonitrile.

In conclusion, we have demonstrated a facile and efficient synthesis of $N^9$-(2-chloroethyl)guanine that avoids the use of chromatography. This compound is a versatile intermediate and can be used to synthesize other $N^9$-substituted guanines via nucleophilic substitution reactions.

## EXPERIMENTAL

### General

All purchased reagents were used without further purification. Thin-Layer Chromatography (TLC) was performed on Selecto Scientific Silica Gel 60, F-254 TLC plates. Melting points were obtained using a Laboratory Devices MEL-TEMP and are uncorrected. $^{1}$H-NMR spectra were obtained using a Varian Gemini-200 (200 MHz). Chemical shifts are reported as δ in ppm. All $^{1}$H-NMR spectra are referenced to the solvent residual peak of DMSO-$d_6$ (δ 2.50) unless otherwise noted.

**Tetrabutylammonium 2-amino-6-chloropurin-9-ide (1).** Procedure was adapted from Bisacchi *et al.* Aqueous tetrabutylammonium hydroxide (~1.5 M, 39.3 mL, ~59 mmol)

was added to a slurry of 2-amino-6-chloropurine (10.0 g, 59 mmol) in 250 mL of $CH_2Cl_2$. The reaction mixture was stirred for 30 min and the solvent removed *in vacuo*. Concentration from toluene (3 x 100 mL) allowed for the removal of water and resulted in crystallization. The solid was triturated with 200 mL of ether, filtered, washed with ether, and dried under vacuum over $CaSO_4$ to yield **1** (24.4 g, 59 mmol, 100%). The orange solid was used without further purification: mp 66-70 °C; $R_f$ = 0.60 ($CHCl_3$:EtOAc:EtOH 1:1:1); $^1$H-NMR ($CDCl_3$, referenced to TMS δ 0.00) δ 0.90 (12H, t), 1.28 (16H, m), 2.90 (12H, m), 7.40 (2H, s), 7.90 (1H, s).

**$N^9$- and $N^7$-(2-chlororoethyl)-2-amino-6-chloropurine (2, 3).** To a solution of **1** (10.0 g, 24 mmol) in 30 mL of $CH_2Cl_2$ was added 1-bromo-2-chloroethane (10 eq, 19.9 mL, 240 mmol). The reaction mixture was stirred at rt for about 1.5 h, during which time precipitation occurred. The solid was filtered, washed with cold $CH_2Cl_2$, and dried under vacuum over $CaSO_4$ to yield a 5:1 mixture of **2** and **3** (3.99 g, 17 mmol, 71% combined yield). Recrystallization from $CH_2Cl_2$ (~20 mL) provided 2.66 g of the $N^9$ isomer, **3**, free of contamination with $N^7$ isomer **2**. The $^1$H-NMR spectrum of **3** was consistent with that reported previously[15]: mp 189-191 °C; $R_f$ = 0.65 ($CHCl_3$:EtOAc:EtOH 1:1:1); $^1$H-NMR (DMSO-$d_6$) δ 4.04 (2H, t), 4.41 (2H, t), 6.99 (2H, s), 8.17 (1H, s). Compound **2**: $R_f$ = 0.49 ($CHCl_3$:EtOAc:EtOH 1:1:1); $^1$H-NMR (DMSO-$d_6$) δ 4.03 (2H, t), 4.67 (2H, t), 6.71 (2H, s), 8.42 (1H, s).

**$N^9$-(2-chloroethyl)guanine (4).** A solution of 3 M $HCl_{(aq)}$ (13 mL), containing **3** (2.5 g, 10.8 mmol), was refluxed for 1 h. The solution was cooled to rt and neutralized with

saturated $NaHCO_{3(aq)}$. The resulting solid was filtered, washed with cold water, and dried under vacuum over $CaSO_4$ to yield **4** (2.31 g, 10.8 mmol, 100%): mp >250 °C; $R_f = 0.24$ ($CHCl_3$:EtOAc:EtOH 1:1:1); $^1$H-NMR (DMSO-$d_6$) δ 3.97 (2H, t), 4.29 (2H, t), 6.53 (2H, s), 7.74 (1H, s), 10.70 (1H, s).

**$N^9$-(2-iodoethyl)guanine (5).** A solution of **4** (100 mg, $4.68 \times 10^{-4}$ mol) containing NaI (10 eq, 701 mg, $4.68 \times 10^{-3}$ mol) in DMF (2-3 mL) was stirred at 75 °C for 1.5 h. The DMF was removed under vacuum. The resulting solid was triturated with water, filtered, washed with water, and dried under vacuum over $CaSO_4$ to yield **5** (110 mg, $3.61 \times 10^{-4}$ mol, 77%): mp >250 °C; $^1$H-NMR (DMSO-$d_6$) δ 3.56 (2H, t), 4.29 (2H, t), 6.49 (2H, s), 7.72 (1H, s), 10.59 (1H, s).

**2-amino-9-(2-(benzylamino)ethyl)-1*H*-purin-6(9*H*)-one (6).** Benzylamine (4 eq, $1.87 \times 10^{-3}$ mol 204 μL) was added to a solution of **4** (100 mg, $4.68 \times 10^{-4}$ mol) containing NaI (21 mg, $1.40 \times 10^{-4}$ mol) in DMSO-$d_6$ (~1 mL). The solution was then stirred for 15 h at 75 °C. After cooling the reaction mixture to rt, acetone (~20 mL) was added, resulting in precipitation. Addition of acetone resulted in precipitation. The solid was filtered, washed with acetone, and dried under vacuum over $CaSO_4$ to yield **6** (47 mg, $1.65 \times 10^{-4}$ mol, 35%): $^1$H-NMR (DMSO-$d_6$) δ 3.24 (2H, t), 4.11 (1H, s), 4.28 (2H, t), 6.66 (2H, s), 7.43 (5H, m), 7.70 (1H, s), 8.77 (1H, s), 10.76 (1H, s).

**2-amino-9-(2-(ethylthio)ethyl)-1*H*-purin-6(9*H*)-one (7).** NaI (21 mg, $1.40 \times 10^{-4}$ mol) and **4** (100 mg, $4.68 \times 10^{-4}$ mol) were added to a solution of NaOH (4 eq, $1.87 \times 10^{-3}$ mol,

75 mg) and ethanethiol (4 eq, 1.87 x $10^{-3}$ mol, 138 μL) in DMSO (1 mL). The resulting solution was stirred at 75 °C for 1 h and then cooled to rt. Water (~20 mL) and glacial acetic acid (0.1 mL) were added until precipitation occurred. The resulting solid was filtered, washed with water, and dried under vacuum over $CaSO_4$ to yield **7** (66 mg, 2.76 x $10^{-4}$ mol, 59%): $^1$H-NMR (DMSO-$d_6$, referenced to HOD δ 3.30) δ 1.09 (3H, t), 2.45 (2H, q) 2.82 (2H, t), 4.05 (2H, t), 6.39 (2H, s), 7.64 (1H, s), 10.51 (1H, s).

**2-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)ethyl benzoate (8).** A solution of **4** (100 mg, 4.68 x $10^{-4}$ mol) containing NaI (21 mg, 1.40 x $10^{-4}$ mol) and sodium benzoate (4 eq, 1.87 x $10^{-3}$ mol, 270 mg) in DMSO-$d_6$ (2-3 mL) was stirred at 120-130 °C for 3.5 h. Water (~20 mL) was added to the hot reaction mixture and a precipitate formed. The solid was filtered, washed with water, and dried under vacuum over $CaSO_4$ to yield **8** (54 mg, 1.80 x $10^{-4}$ mol, 39%): $R_f$ = 0.32 ($CHCl_3$:EtOAc:EtOH 1:1:1); $^1$H-NMR (DMSO-$d_6$) δ 4.36 (2H, t), 4.56 (2H, t), 6.47 (2H, s), 7.50 (2H, t), 7.65 (2H, t), 7.77 (1H, s), 7.91 (2H, d), 10.58 (1H, s).

**2-amino-9-vinyl-1*H*-purin-6(9*H*)-one (9).** A solution of **4** (100 mg, 4.68 x $10^{-4}$ mol) containing NaI (21 mg, 1.40 x $10^{-4}$ mol) in DMSO (1 mL) was treated with NaOH (4 eq, 1.87 x $10^{-3}$ mol, 75 mg) and stirred at 75-85 °C for 1.5 h. The resulting precipitate was cooled to rt and dissolved in water (~5 mL). Precipitation occurred following addition of glacial acetic acid (~1 mL). The purple solid was filtered, washed with water, and dried under vacuum over $CaSO_4$ to yield **9** (68 mg, 3.84 x $10^{-4}$ mol, 82%): $^1$H-NMR (DMSO-$d_6$) δ 5.02 (1H, d), 5.85 (1H, d), 6.57 (2H, s), 7.05 (1H, q), 8.08 (1H, s), 10.73 (1H, s).

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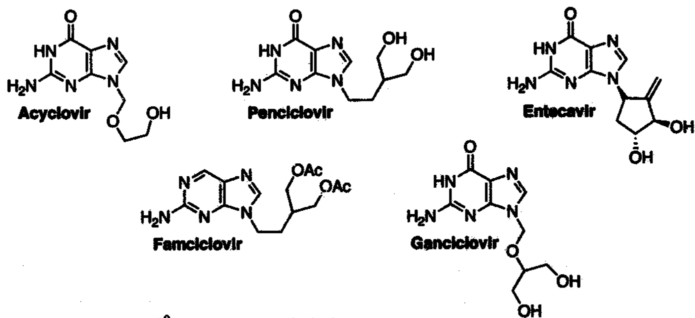
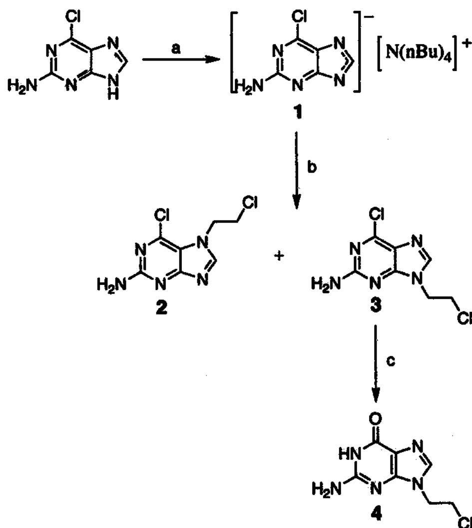
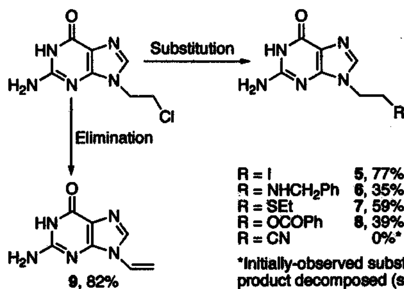



***Figure 1:*** various $N^9$-substituted anti-virals.




***Scheme 1.*** Reagents and Conditions: (a) 1.5 M TBAH, $CH_2Cl_2$, rt, 30 min (b) $Br(CH_2)_2Cl$, $CH_2Cl_2$, rt, 1.5 h (c) 3 M $HCl_{(aq)}$, reflux, 1 h.

*Scheme 2.*



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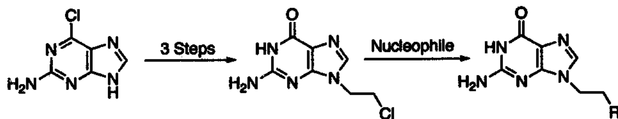
*Efficient Synthesis of $N^9$-alkylguanines*

Graphical Abstract:

An efficient synthesis of $N^9$-(2-chloroethyl)guanine from 2-amino-6-chloropurine is reported. Subsequent displacement reactions allowed for the preparation of various $N^9$-substituted guanines.



# *Evaluation of a More Highly Conjugated Analog of Guanidinium-Rich Molecular Transporters Using a pH Dependent Fluorescence Assay*

**David E. Olson[a], Barbara Danowski[b], and Joanne D. Kehlbeck[a]***

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**Abstract:**

The importance of the guanidino functional group in the cellular uptake of guanidinium-rich molecular transporters has been thoroughly demonstrated. Its ability to act as a bidentate hydrogen bond donor appears to play a key role in this process, but the effect of polarizability on cellular internalization has not been determined. We report the synthesis of a peptoid molecular transporter containing more highly conjugated guanine headgroups and its subsequent evaluation using a pH dependent fluorescence assay.

**Introduction:**

An important role of biological membranes is to exclude harmful substances from the interior of the cell. However, this defense mechanism also prevents the passive cellular uptake of many useful therapeutics. Currently, the design of drugs limits their physical properties to a very narrow range of hydrophobicities, as they must be both soluble in

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polar extracellular fluids and sufficiently nonpolar to passively diffuse through the lipid bilayer of the cell. These constraints impose limitations on the design of therapeutics, and consequently, may hinder their effectiveness.

A class of compounds, collectively known as molecular transporters, has shown promising results in the area of drug delivery.[1] Many of these molecules are derived from natural occurring proteins capable of traversing the cellular membrane.[2-6] In particular, the nuclear transcription activator protein (Tat) encoded by HIV-1 has the ability to cross the plasma membrane, and its highly basic region, consisting of residues 49-57, has been shown to be primarily responsible for its cellular uptake.[7-11]

Analogs of this highly basic region enter cells by a mechanism that is currently unresolved.[12-19] They typically consist of cationic residues that serve to increase the water solubility of their molecular cargo. Therefore, these molecular transporters increase the bioavailability of existing drugs as well as allow new drugs to be designed without the structural limitations that have hindered the development of current therapeutics.

Various molecular transporters have been shown to effectively traverse cellular membranes despite their structural dissimilarities. Nonnatural D-peptides[20], as well as molecular transporters containing peptoid[20] or oligocarbamate[21] backbones have all been shown to efficiently enter cells. Also, use of aminocaproic acid spacers between residues[22] and an increase in the side chain lengths[20] improved cellular uptake. This

broad range of structural features appears to be inconsistent with a receptor-mediated process. However, the guanidinium moiety is a common structural feature in all of these molecular transporters.

The homopolymers of lysine, ornithine, and histidine, cationic transporter molecules that lack the guanidinium group, have been shown to cross the lipid bilayer of the cell with only limited success.[23-24] However, arginine homopolymers are internalized very effectively.[24] This suggests that it is the unique properties of the guanidinium group that allow for the efficient cellular uptake of guanidinium-rich molecular transporters.

Rothbard *et al.* have proposed that it is the ability of the guanidinium group to act as a bidentate hydrogen bond donor that enables guanidinium-rich molecular transporters to be internalized.[15] Presumably, the positively charged guanidinium group would associate with cell surface moieties such as sulfates or phosphates through a bidentate hydrogen bond interaction. This less polar ion pair would then be able to diffuse through the nonpolar lipid bilayer of the cell.

The current study seeks to explore another unique feature, namely the polarizability of the guanidinium group, that might also contribute to the effectiveness of guanidinium-rich molecular transporters. Cellular uptake studies were performed using a molecular transporter rich in guanine, a more highly conjugated analog of the guanidino group. We hypothesized that this increase in conjugation, leading to an increase in polarizability, might affect the extent to which this new transporter is internalized.

Also, by exploiting the pH sensitivity of fluorescein[25-28], we hoped to clarify the extent to which the fluorescent conjugates of molecular transporters are truly internalized. It has been shown that the emission of fluorescein can be selectively quenched on the exterior of cells following a lowering of the local pH[29]. This procedure does not affect the intensity of fluorescence resulting from internalized fluorescein and can therefore be used to qualitatively compare the amount of fluorescent probe that has been internalized to that which has merely adhered to the cell surface. Most studies to date involving molecular transporters have used flow cytometry to monitor internalization and have had faith that numerous washes removed all externally adhered transporter. This pH dependent fluorescence assay provides a means for qualitatively determining the fluorescence due to cell surface adhesion.

## Experimental:

**General.** Rink amide-MBHA resin was purchased from NovaBiochem, while 2-amino-6-chloropurine was purchased from AB CHEM TECHNOLOGIES, LLC. All other reagents were purchased from Aldrich and used without further purification.

**Fl-aca-*N*-hxg$(NH_2)_9$-$CONH_2$ (1).** A modified procedure based on a microwave assisted[30] sub-monomer approach to peptoid synthesis[31-33] was employed. Peptoids were manually synthesized in fritted vessels using mechanical agitation to ensure mixing of the resin. A chloranil test for secondary amines was performed at each stage of the synthesis

in order to evaluate the progress of the reactions.[34] Fmoc protected Rink amide resin (100 mg) was agitated in DMF (4-5 mL) for 30 min and afforded approximately 50 μmol of exposed amine functional groups following treatment with 20% (v/v) piperidine/DMF (1-2 mL for 1 min, 2-3 mL for 10 min, and 2-3 mL for 10 min). The resin was then washed with DMF (2-3 mL x 5) before being treated with a solution of bromoacetic acid (20 eq) and DIC (26 eq) in 4 mL of DMF. The reaction mixture was microwaved at 30% (2 x 15 s with manual agitation in between) using a Panasonic NN-S443 microwave oven and agitated for an additional 45 mins. Next, the resin was washed with DMF (2-3 mL x 5) and treated with a solution of DMF (4 mL) containing *N*-Boc-1,6-hexanediamine (17 eq). The reaction mixture was microwaved at 30% (2 x 15 s with manual agitation in between) and agitated for an additional 45 mins. The resin was then washed with DMF (2-3 mL x 5). The bromoacetic acid coupling and amine substitution steps were repeated until a 9-residue oligomer had been obtained. After the addition of 6-(Fmoc-amino)caproic acid (20 eq) and DIC (26 eq) in DMF (4 mL), the reaction mixture was microwaved at 30% (2 x 15 s with manual agitation in between). The vessel was then agitated for an additional 45 mins. Conjugation of fluorescein-5-isothiocyanate (0.2 mmol) to the transporter molecule in the presence of DIEA (2 mmol) was complete after a 15-hour incubation period. Care was taken to avoid unnecessary exposure of the fluorescein moiety to light. Next, the resin was drained of excess fluorophore and washed with DMF (3 x 5 mL) and dichloromethane (5 x 5 mL). The resin was then air dried by applying a vacuum for 10 min. Cleavage from the resin was achieved using a 95:5 (v/v) TFA/triisopropylsilane cocktail mixture. After 4 hours the product was collected and the TFA was removed *in vacuo*. The crude oil was triturated with cold

ether (20 mL) and centrifuged. The ether was removed by decantation. The resulting orange solid will be purified by RP-HPLC, isolated by lyophilization, and characterized by electrospray mass spectrometry. The concentration of 1 was determined from a Beer's Law plot using the extinction coefficient of fluorescein ($\varepsilon$ = 72,000 $M^{-1}$ $cm^{-1}$, 494 nm).

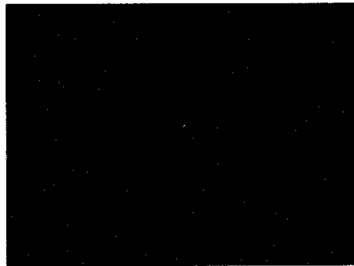
**Fl-aca-*N*-hxg(Guanidinium)$_9$-CONH$_2$ (2).** Perguanidinylation of **1** by the method of Wender *et al.*[20] afforded **2**, which will be purified by RP-HPLC, isolated by lyophilization, and characterized by electrospray mass spectrometry. The concentration of **2** was determined from a Beer's Law plot using the extinction coefficient of fluorescein ($\varepsilon$ = 72,000 $M^{-1}$ $cm^{-1}$, 494 nm). However, care was taken to ensure that the solution of **2** was neutral before absorbance measurements were taken.

**Fl-aca-*N*-hxg(Guanine)$_9$-CONH$_2$ (3).** The procedure for the synthesis of **1** was followed with the exception that *N*-Boc-1,3-propanediamine was used in place of *N*-Boc-1,6-hexanediamine. Next, the peptoid will be treated with $N^9$-(2-chloroethyl)guanine and NaI (30 mol %) in DMF. $N^9$-(2-chloroethyl)guanine was prepared by the method of Olson *et al.*[35] Compound **3** will be purified by RP-HPLC, isolated by lyophilization, and characterized by electrospray mass spectrometry. The concentration of **3** will be determined from a Beer's Law plot using the extinction coefficient of fluorescein ($\varepsilon$ = 72,000 $M^{-1}$ $cm^{-1}$, 494 nm).

## Preliminary Results:

Live Swiss 3T3 mouse embryo fibroblast cells were incubated in OPTIMEM containing either 2 μM of **1** (negative control) or **2** (positive control) for 60 min at 37 °C, and the results are shown in Figure 1. Very little cellular uptake of **1** is observed, as expected. Compound **2** seems to be readily internalized and appears to be localized in endocytic vesicles.

**Negative Control (1)**



**Positive Control (2)**

**Figure 1:** Swiss 3T3 mouse embryo fibroblast cells incubated with either 2 μM of compound **1** or **2**.

A fluorescence quenching assay will also be employed in this study. The fluorescence of fluorescein will be quenched following addition of acid to the incubation media. This procedure should not affect internal pH. However, a control study was performed to assess the effectiveness of this pH dependent fluorescence quenching assay. Swiss 3T3 mouse embryo fibroblast cells were incubated with free fluorescein isothiocyanate (FITC). Fluorescent images were taken using confocal microscopy prior to and following the addition of acid to the incubation media. The results can be seen in Figure

2. Fluorescent signal observed after the addition of acid is presumably a result of internalized fluorophore while that observed prior to the addition of acid is a result of both internalized fluorophore and that which has adhered to the cell surface.

**pH = 7.35** **pH = 6.50**

Figure 2: Swiss 3T3 mouse embryo fibroblast cells incubated with FITC (pre- and post-addition of acid to the incubation media).

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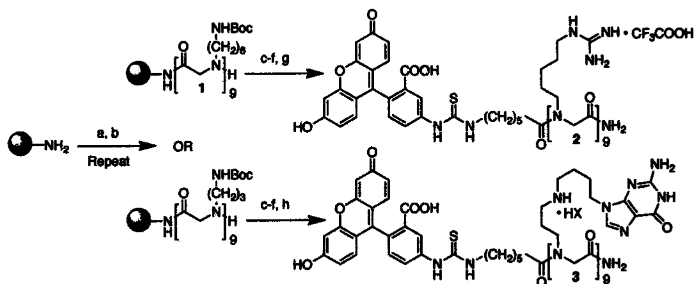


***Scheme 1.*** Reagents and Conditions: (a) bromoacetic acid, DIC, DMF (b) *N*-Boc-1,6-hexanediamine or *N*-Boc-1,3-propanediamine, DMF (c) 6-(Fmoc-amino)caproic acid (d) piperidine, DMF (e) FITC, DIEA, DMF (f) 95:5 TFA/TIS (g) pyrazole-1-carboxamidine hydrochloride, $Na_2CO_3$ (h) $N^9$-(2-chloroethyl)guanine, NaI, DMF.

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David E. Olson
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