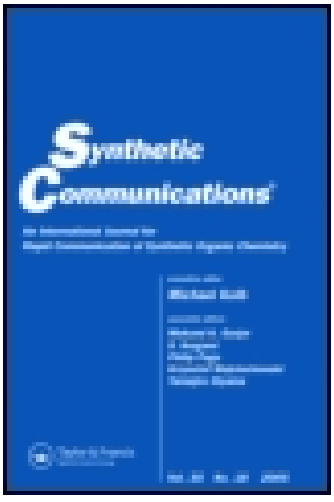

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Convenient Synthesis of an N-(1-Alkoxyl-9-fluorenyl)serine Acridine Conjugate

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Convenient Synthesis of an *N*-(1-Alkoxyl-9-fluorenyl)serine Acridine Conjugate

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Abstract: We report here the synthesis of an *N*-(1-alkoxyl-9-fluorenyl)serine acridine conjugate, which was achieved by a three-component assembly approach via an intramolecular reductive amination process.

Keywords: acridine conjugate, 1-alkoxyl-9-fluorenylamine, serine

9-Fluorenylamines have recently been demonstrated as important chemophores for biological activity in a variety of organic compounds.[1–4] For example, hydroxylated or methoxylated 9-fluorenylamino-pentanenitriles have potent anti-multidrug-resistant activity for cancer therapy,[3] and 1-hydroxyfluorenyl dinitrophenyl hydrazone is highly cytotoxic in breast, ovary, colon, and melanoma cancer cells.[4] The synthesis of 9-fluorenylamino derivatives has been accomplished either through reductive amination of 9-fluorenones,[3] or nucleophilic substitution of 9-bromofluorene by amines.[5] However, in the synthesis of α-substituted amino acid derivatives of 1-alkoxy(or hydroxyl)-fluorenylamine for potential drug candidates, we found that neither method produced desired products. Unsuccessful attempts of reductive amination were probably due to steric interaction between α-amino acids and 1-substituted fluorenones, and basic conditions for SN2-type reactions were not compatible with the benzyl ester of amino acid derivatives. Thus, an alternative synthetic approach was needed. We report here a

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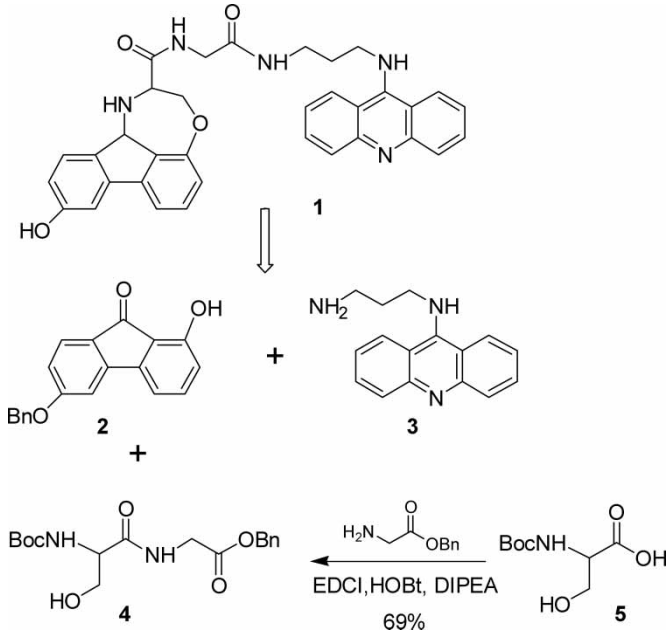
convenient synthetic approach via an intramolecular reductive amination process to obtain 1-alkoxyl-9-fluorenylaminoserine acridine, conjugate **1** (Scheme 1). In addition, this method could be easily modified to conjugate with other bioactive chemophores besides acridine which is a DNA intercalator and is used in this study for potential additional biological activity.[6]

The synthetic design involved the coupling of serine derivative **4** and fluorenone **2** through an ether linkage to facilitate the reductive amination process (Scheme 1). Acridine **3** was conjugated at later synthetic steps. Initially, the benzyl ester of serine without glycine was used but was found not to be able to couple with fluorenone **2** in the subsequent Mitsunobo step. Thus, *t*-Boc-serine-glycine benzyl ester **4** was used instead, which was conveniently synthesized by coupling *t*-Boc-protected serine **5** and glycine benzyl ester using 1-(3-dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) in 69% yield.

9-Fluorenone **2** was synthesized via a Suzuki coupling, followed by an acid-catalyzed acylation (Scheme 2). Boronic acid-**7** for the Suzuki coupling was obtained from bromide **6** and was used immediately in the Suzuki reaction because of its high instability.[7,8] Triflate **8** was synthesized using reported procedures.[8] For the Suzuki coupling of **7** and **8**, we found that a combination of Cs_2CO_3 and THF afforded a higher yield (88%) than other conditions including Na_2CO_3, K_3PO_4, dioxane, or acetonitrile (<45%). Hydrolysis of the resulting biphenyl **9** to a free acid was carried out using



Scheme 1. Synthetic design of fluorenylaminoserine acridine conjugate **1**.

Scheme 2. Conditions and reagents: (i) nBuLi, THF, −78°C then B(O-*i*-Pr)$_3$; (ii) triflate **8**, Pd(PPh$_3$)$_4$, KBr, Cs_2CO_3, THF-H_2O, reflux for 16 h; (iii) (a) NaOH, THF-H_2O; (b) TFA, TFAA, 0°C; (iv) PPh$_3$, **4**, DIAD, THF, sonication; (v) (a) TFA; (b) silica gel, reflux in CH_3CN, 16 h; (c) $NaBH_3CN$; (vi) (a) 5% HCOOH in MeOH, Pd/C; (b) acridine **3**, HBTU, DIPEA, DMF, 3 h.

NaOH in a mixed THF–water solution. Acid-catalyzed acylation was achieved with trifluoroacetic acid and anhydride (2:1 ratio) at 0°C, affording fluorenone **2** as the major isomer in 72% yield. The structure of fluorenone **2** was confirmed by the observed coupling pattern of a singlet and four doublets in the aromatic region of the ^{1}H NMR spectrum.

Initial attempts to couple serine **4** and fluorenone **2** under normal Mitsunobu reaction conditions[9] with diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD) produced no desired product **10**. Recently, Lepore and He reported that under sonication, Mitsunobu reactions of steric hindered alcohols proceeded smoothly at high concentrations.[10] Indeed, we found that the coupling was successful only under this specific condition with serine derivative **4** in 40% yield. The subsequent intramolecular reductive amination of 9-fluorenone **10** was accomplished first by the removal of the *t*-Boc protecting group at 0°C, then the formation of imine through refluxing with silica gel, and finally the reduction with $NaBH_3CN$.[11] A comparable yield was obtained when 3-Å molecular sieves were used as the dehydrating agent.

For incorporation of the acridine moiety, the benzyl protecting groups of 9-aminofluorene **11** were removed by Pd/C in a 5% HCOOH solution. Acridine **3** was coupled through an amide linkage using O-benzotriazol-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and N,N-diisopropylethylamine (DIPEA) in DMF (Scheme 2). The progress of the reaction was monitored by high performance liquid chromatography (HPLC) analysis. Conjugate **1** was finally isolated in 24% yield by HPLC separation using a gradient condition of 10–70% CH_3CN in a triethylamine acetate buffer (pH 6) over 30 min. Conjugate **1** was confirmed by both ^{1}H and ^{13}C NMR analyses and showed a molecular ion of 574.00 m/z for $M + H^+$ (calculated for $C_{34}H_{32}N_5O_4$, 574.25) in the ESI-MS analysis.

In conclusion, the synthesis of 1-alkoxyl-9-fluorenylaminoserine acridine was accomplished by a three-component assembly approach through an intramolecular reductive amination process. We are currently investigating the potential biological activity of this novel fluorenylamine compound.

EXPERIMENTAL PROCEDURES

Compound **4**: DIPEA (314 mg, 2.43 mmol), *t*-butoxycarbonylserine (500 mg, 2.39 mmol), HOBt (395 mg, 2.92 mmol), and EDCI (489 mg, 2.55 mmol) were added to **5** (863 mg, 2.43 mmol) in 20 mL of CH_2Cl_2. The resulting reaction was stirred under N_2 for 16 h and worked up with CH_2Cl_2. Upon concentration, an analytically pure product (587 mg) was obtained in 69% yield as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42–7.34 (m, 5H), 7.20 (br s, 1H), 5.63 (d, $J = 7.2$ Hz, 1H), 5.16 (s, 2H), 4.22 (br s, 1H), 4.15–**08** (m, 3H), 3.79–3.67 (m, 1H), 1.44 (s, 9H). ESI-MS calcd. for $C_{17}H_{25}N_2O_6$ ($M + H^+$) 353.17; found 353.03.

Compound **9**: Compound **7** (387 mg, 1.69 mmol), $Pd(PPh_3)_4$ (90 mg, 0.08 mmol), KBr (146 mg, 1.22 mmol), and Cs_2CO_3 (590 mg, 1.81 mmol) were added to **8** (395 mg, 1.21 mmol) in dry THF (18 mL). The mixture was stirred for 15 min, and then 0.5 mL of water was added. The resulting mixture was refluxed for 16 h. The reaction was worked up with brine and ether. The desired product **9** (383 mg) was purified by a flash column in 88% yield as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.63 (t, $J = 7.7$ Hz, 1H), 7.58 (d, $J = 6.8$ Hz, 2H), 7.52 (t, $J = 7.2$ Hz, 2H), 7.45 (t, $J = 7.7$ Hz, 2H), 7.14–7.05 (m, 5H), 5.21 (s, 2H), 1.91 (s, 6H). ESI-MS calcd. for $C_{23}H_{21}O_4$ ($M + H^+$) 361.14; found 361.07.

Compound **2**: NaOH (70 mg) and 0.5 mL water were added to **9** (94 mg, 0.26 mmol) in 10 mL of THF. The reaction mixture was refluxed for 16 h and then acidified to pH 4–5 with diluted HCl. The reaction was worked up with ether. Upon concentration, the resulting residue was dissolved in 5 mL of CH_2Cl_2. Trifluoroacetic acid (2 mL) and trifluoroacetic anhydride (1 mL) were then added at 0°C. After 2 h, the reaction was worked up with ether. The desired product **2** (56.4 mg) was purified by a flash column in 72% yield as a yellow solid (mp 103–104°C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.48 (s, 1H), 7.58 (d, $J = 8.3$ Hz, 1H), 7.46–7.31 (m, 6H), 7.10 (s, 1H), 6.98 (d, $J = 7.2$ Hz, 1H), 6.81 (d, $J = 7.8$ Hz, 1H), 6.76 (d, $J = 8.6$ Hz, 1H), 5.16 (s, 2H). ESI-MS calcd. for $C_{20}H_{15}O_3$ ($M + H^+$) 303.10; found 302.99.

Compound **10**: Serine **4** (300 mg, 0.85 mmol) and PPh_3 (260 mg, 0.99 mmol) were added to **2** (200 mg, 0.66 mmol) in dry THF (0.8 mL). The mixture was sonicated for 15 min, and then DIAD (143 μL, 0.73 mmol) was added under sonication. The reaction mixture was sonicated for another 40 min and worked up with CH_2Cl_2. The desired product (168 mg) was purified by a flash column in 40% yield as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.40 (br s, 1H), 7.52 (d, $J = 8.2$ Hz, 1H), 7.47–7.34 (m, 6H), 7.26–7.22 (m, 5H), 7.12–7.09 (m, 2H), 6.81 (d, $J = 2.2$ Hz, 1H), 6.78 (d, $J = 8.4$ Hz, 1H), 5.90 (br s, 1H), 5.16 (s, 2H), 5.06 (d, $J = 4.1$ Hz, 2H), 4.62 (br s, 2H), 4.25 (dd, $J_1 = 6.7$ Hz, $J_2 = 17.6$ Hz, 1H), 4.10 (dd, $J_1 = 5.30$ Hz, $J_2 = 17.6$ Hz, 1H), 3.82 (br t, $J = 8.6$ Hz, 1H), 1.46 (s, 9H). ESI-MS calcd. for $C_{37}H_{37}N_2O_8$ ($M + H^+$) 637.25; found 637.00.

Compound **11**: Trifluoroacetic acid (1 mL) was added to **10** (90 mg, 0.14 mmol) in dry CH_2Cl_2 (2 mL) at 0°C. After 3 h, the solvent was removed by vacuum. The resulting brown residue was dissolved in a mixture of 3 mL of CH_2Cl_2 and 9 mL of CH_3CN, followed by the addition of 460 mg of silica gel. The resulting mixture was refluxed for 16 h under N_2. After cooling to room temperature, $NaBH_3CN$ (96 mg, 1.5 mmol) was added. The reaction was stirred for 2 h, and acetic acid (170 mg) was added. After 3 h, the reaction was worked up with CH_2Cl_2. The desired product (34 mg) was purified by a flash column in 47% yield as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.94 (br s, 1H), 7.58 (d, $J = 8.2$ Hz, 1H), 7.48–7.26 (m, 13 H), 6.95 (d, $J = 7.4$ Hz, 2H), 5.27 (d, $J = 13.2$ Hz, 1H), 5.17 (s, 2H), 5.15 (s, 2H), 4.85 (s, 1H), 4.17–4.10 (m, 3H), 3.64 (br t,

$J = 12.0$ Hz, 1H). ESI-MS calcd. for $C_{32}H_{29}N_2O_5$ ($M + H^+$), 521.21; found 520.97.

Compound **1**: To **11** (20 mg, 0.038 mmol) in 5% HCOOH in MeOH (2 mL), 10% Pd/C (80 mg) was slowly added under N_2. After 20 min, the solid was removed with a 50 μm filter, and then 0.5 mL of trifluoroacetic acid was added to the filtrate. Upon concentration, the free acid (11 mg) was obtained. For the coupling, acridine **3** (14 mg, 0.04 mmol), HBTU (41 mM in DMF, 1.1 mL), and DIPEA (41 mM in DMF, 2.6 mL) were added to the resulting free acid (5 mg, 0.015 mmol) in DMF (1 mL). After 3 h, the desired product **1** (5.3 mg) was purified by a HPLC separation using a Microsorb MV C18 column (10 × 250 mm, 8 μm) from Varian (Walnut Creek, CA) in 24% yield as a yellow oil. ^{1}H NMR (MeOH-d4, 300 MHz): δ 8.37 (d, $J = 6.6$ Hz, 2H), 7.83 (d, $J = 6.3$ Hz, 2H), 7.74 (t, $J = 6.6$ Hz, 2H), 7.50 (d, $J = 6.0$ Hz, 1H), 7.41 (t, $J = 5.7$ Hz, 2H), 7.35 (d, $J = 5.4$ Hz, 1H), 7.24 (t, $J = 5.7$ Hz, 1H), 7.12 (s, 1H), 6.84 (d, $J = 5.7$ Hz, 1H), 6.74 (d, $J = 6.0$ Hz, 1H), 4.97 (d, $J = 10.5$ Hz, 1H), 4.81 (s, 1H), 4.07 (d, $J = 5.4$ Hz, 1H), 3.98 (t, $J = 4.8$ Hz, 2H), 3.87 (d, $J = 4.2$ Hz, 2H), 3.74–3.65 (m, 1H), 3.43–3.37 (m, 2H), 2.29–1.96 (m, 2H). ESI-MS calcd. for $C_{34}H_{32}N_5O_4$ ($M + H^+$) 574.25; found 574.00.

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