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An improved synthesis of arsenic–biotin conjugates

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Abstract—An amide linked conjugate of *p*-aminophenylarsine oxide and biotin is conveniently prepared in a one-pot procedure by the reaction of biotinyl chloride, formed in situ, with *p*-aminophenyldichloroarsine. The reaction of the arsine oxide–biotin conjugate with 1,2-ethanedithiol produces the stabilized dithiarsolane. These reagents are now readily available for a variety of applications.
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1. Introduction

Arsenic compounds have been used as cancer chemotherapeutic drugs for several years. Arsenic and arsenical compounds induce apoptosis in many types of cancer cells and may affect cellular functions directly by interacting with cellular structures, or indirectly by altering the signal transduction pathways and the expression of numerous genes. Direct binding of arsenic is believed to be important in at least two aspects: (1) inhibition of enzymes and perturbation of other proteins may cause chemotherapeutic effects; (2) binding to certain proteins may be a detoxification process.[1] Trivalent arsenic binds many proteins, and the biological responses have been suggested to result from the formation of adducts between closely spaced SH groups of Cys residues.[2] This may inhibit the functions of the target protein.[3]

Among the different compounds of trivalent arsenic, the arylarsine oxides have been studied particularly extensively for over 90 years because of their wide range of biological effects and uses.[2b] A few applications have been the study of active sites of purified proteins,[4] purification of proteins with vicinal dithiols,[5] investigation of signaling pathways in T-cell cytotoxicity,[6] blocking ubiquitin-dependent protein degradation,[7] interfering with insulin-activated hexose transport,[8] and others.

Biotin is a water-soluble member of the B-complex group of vitamins and is commonly referred to as vitamin H. The use of biotinylated bifunctional reagents is well documented, and the biotin–avidin complex has been used for a variety of biomedical applications. These include targeted radiotherapy,[9] tumor-targeted probes for dual-modality magnetic resonance and fluorescence imaging,[10] bioanalytical chemistry,[11] immunology,[12] studies of receptors on the surface of tumour cells,[13] and others. In the same way, the complex with streptavidin has been used as an attractive model for studying protein–ligand interactions,[14] in determination of amino acid residues involved in ligand binding at the NBMPR-binding site of the ENT1 nucleoside transporter,[15] and in the immobilization of DNA.[16] Also, these complexes are used in pharmacology for controlled delivery and uptake of ligands.[17]

The arsenic–biotin conjugate **5** combines the characteristics of biotin and an arsenic reagent and therefore is bifunctional for thiols and avidin (or streptavidin). This conjugate has been used in the study of *Torpedo* nicotinic receptors[18] and recently, for the identification of arsenic-binding proteins in human breast cancer cells.[19] Other complexes that present both the structures (arsenic and biotin) were used to identify closely spaced thiols in cell-surface proteins of mammalian cells.[20]

We report herein an improved synthesis of arsenic–biotin conjugate **5** from biotin. This conjugate in turn will be used to selectively label the mitochondrial targets in a way that facilitates purification and detection.

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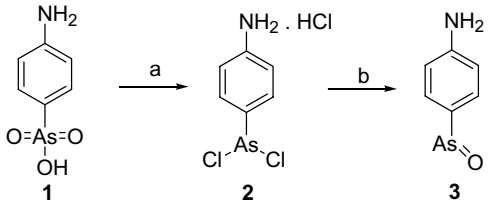
2. Results and discussion

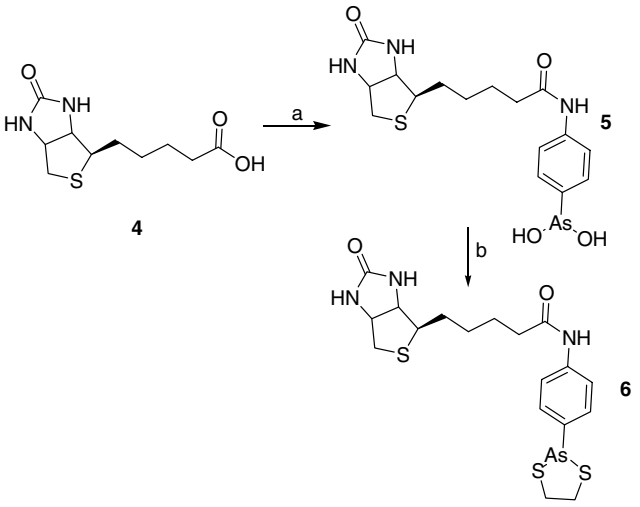
In our initial attempts to synthesize **5** by the previously reported procedure,[19] we used the commercially available 4-nitrophenyl biotin ester as a starting material. The *p*-aminophenylarsine oxide (**3**) was synthesized in 53% yield from *p*-arsanilic acid (**1**) by a published procedure (Scheme 1).[23] The reaction between 4-nitrophenyl biotin ester and **3** produced a complex mixture of products, and the arsenic–biotin conjugate **5** was detected only in trace amounts. To our surprise, an arsenic-free biotin conjugate was obtained in 16% yield. The apparent low reactivity of the 4-nitrophenyl biotin ester together with low nucleophilicity of aminophenylarsine oxide could play an important roll in the low yield since there was an evidence for the decomposition of the oxide under the reactions conditions. Based on this, the use of a more reactive biotin compound would be beneficial. In fact, the reaction with biotinoyl chloride is a reasonable option, but even here this reported procedure using the chloride afforded **5** in only 14% yield.[18]

Despite the fact that *p*-aminophenylarsine oxide (**3**) exists as hydrate,[21] with time it probably no longer exists as a monomer. Other aryl arsenoxides have been reported to form an eight membered As_4O_4 ring and to exist as cycles $(Ar–AsO)_4$.[22] For this reason freshly synthesized material must be used in the reaction with biotin.[18] Considering this, we explored the use of the more stable arsine oxide precursor **2**. The *p*-aminophenyldichloroarsine·HCl (**2**) was synthesized in 98% yield from *p*-arsanilic acid (**1**) according to the published procedure (Scheme 1).[23]

The synthesis of amides directly from amines and carboxylic acids by in situ preparation of an activated acyl groups is well precedented.[24] The reaction between biotin (**4**) and **2** via biotinoyl chloride is an attractive option for two reasons. First, this minimizes the number of steps which should increase the overall yield of **5**. Second, this uses directly the commercially available and inexpensive biotin (**4**). With this in mind, we explored the synthesis of **5** by the reaction of amine **2** with biotinoyl chloride generated in situ from biotin and ethyl chloroformate (Scheme 2).[25] We found that the solutions of arsine **2** decomposed in THF and 1,4-dioxane (the solution change from colorless to orange), while it is not soluble in ether. The decomposition is almost immediate in THF. However, in methanol there was no visible change in the solution and TLC indicated that arsine **2** was stable in this solvent.



Scheme 1. Reagents and conditions: (a) MeOH, HCl, KI, SO_2, rt, 30 min; (b) 10% NH_4OH, rt, 15 min.



Scheme 2. Reagents and conditions: (a) i—CH_2Cl_2, triethylamine, ethyl chloroformate, −5 °C, 5 h; ii—**2**, triethylamine, MeOH, rt, 3 days; iii—2 N NH_4OH, rt, 10 min; (b) MeOH, 1,2-ethanedithiol, rt, 1 h.

The reaction between the biotin and ethyl chloroformate was performed in CH_2Cl_2 at −5 °C for 5 h to generate the acyl chloride. A methanolic solution of **2** was then added, and after 3 days of reaction and subsequent treatment with NH_4OH to convert the arsine dichloride to the oxide, essentially complete conversion to **5** was indicated by TLC. A chromatographic purification affords the arsenic–biotin conjugate **5** in 37% yield. The low yield may reflect limited solubility of **2** in CH_2Cl_2. The use of acetonitrile, a more polar solvent, to prepare the acyl chloride increased the yield of **5** to 50% after two days of reaction. The ^1H NMR and MS were in complete agreement with the data reported.[18]

The solutions of arsenoxides exist as hydrates and can be deactivated over time, presumably by the oxidation of arsenic from the trivalent to the pentavalent state.[20] To prevent this, the As–$(OH)_2$ group of **5** can be protected by reaction with dithiols. In addition, it has been reported that the attachment of dithiol ligands can greatly enhance the trypanocidal activity of certain arsenoxides.[26] This suggests that the use of a dithiol ligand could be an additional strategy to improve the selectivity of drug access to the target. With this in mind, we protected the arsenic moiety of the biotin–arsenic conjugate **5** using 1,2-ethanedithiol to form the pure dithiarsolane **6** in 86% yield.

3. Conclusion

In summary, the biotin–arsenic conjugate **5** was synthesized in a total yield of 49% from biotin and *p*-aminophenyldichloroarsine **2**. Although the isolated yield was moderate, it is higher that the previously reported.[18,19] Major advantages include the ready availability of starting materials **2** and biotin. With this new preparation procedure of **5** on a gram-scale can be done conveniently, avoiding the use of expensive activated esters of biotin. In addition, dithiarsolane **6**, ob-

tained in a high yield by direct reaction of **5** with 1,2-ethanedithiol, provides the biotin conjugate in a stabilized form.

4. Experimental

4.1. General

All solvents and reagents were from Aldrich and used without further purification. NMR spectra were run in CD_3OD or DMSO-d_6 on a Varian Mercury 300 MHz spectrometer. Chemical shifts are expressed in ppm with TMS as an internal reference. Mass spectra were determined using a Hewlett Packard 1100 MSD instrument. Reactions were monitored by TLC on silica gel using 10% methanol in dichloromethane as a solvent and compounds visualized by UV lamp. Flash chromatography was carried out in a Biotage SP4™ Purification System. The reported yields are for the purified material and are not optimized.

4.2. Synthesis of *p*-aminophenyldichloroarsine·HCl (2)[23]

To a solution containing methanol (30 mL), HCl (24 mL), and potassium iodine (100 mg) was added *p*-arsanilic acid (**1**) (10.90 g). Sulfur dioxide was bubbled through the stirred solution for 30 min until the solution's color changed from orange to pale yellow. The solution was cooled in ice and the precipitate was collected and washed with cold ethyl ether to afford 13.43 g of *p*-aminophenyldichloroarsine·HCl (**2**) as a white solid. The product was used in the next reaction without further purification. White solid; 98% yield; mp = 128–130 °C (d); ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.98 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ ppm 124.47, 133.23, 134.20, 149.79; HRMS (AP) m/z: calcd for $C_6H_7NCl_2As$ ($M+H^+$-HCl): 237.9172, found: 237.9183.

4.3. Synthesis of *N*-(4-arsenosophenyl) hexahydro-2-oxo-(3a*S*,4*S*,6a*R*)-1*H*-trieno[3,4-*d*]imidazole-4-pentamide (5)

A suspension of biotin (**3**) (336.9 mg, 1.3652 mmol) in acetonitrile (50 mL) at −5 °C was treated with triethylamine (0.19 mL, 1.3564 mmol) and ethyl chloroformate (0.13 mL, 1.3235 mmol). The suspension was stirred at that temperature for 5 h. To a suspension of *p*-aminophenyldichloroarsine·HCl (**2**) (375.5 mg, 1.3684 mmol) in acetonitrile/MeOH 10:1 (11 mL) was added triethylamine (0.38 mL), and this suspension was dropped slowly into the biotin suspension. The reaction mixture was stirred at −5 °C, then warmed to room temperature and stirred for two days. The solvent was removed under vacuum and 2 N NH_4OH (6 mL) was added. The suspension was stirred for 10 min, and cold water (15 mL) was added. The suspension was cooled in ice, and the solid was collected by filtration, washed with cold water and then dried. After column chromatography (KP-SIL column, 2–20% of MeOH in dichloromethane, UV 220–245 nm) 291.1 mg of **5** was obtained. White solid; 50% yield; mp 224–226 °C (lit.,[18] 223–224 °C); ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.58–1.44 (m, 2H), 1.68–1.59 (m, 1H), 1.85–1.68 (m, 3H), 2.41 (t, J = 7.3 Hz, 2H), 2.70 (d, J = 12.7 Hz, 1H), 2.93 (dd, J = 12.8 Hz, J = 4.9 Hz, 1H), 3.22 (ddd, J = 8.8 Hz, J = 6.0 Hz, J = 4.5 Hz, 1H), 4.31 (dd, J = 7.9 Hz, J = 4.5 Hz, 1H), 4.49 (ddd, J = 8.0 Hz, J = 4.9 Hz, J = 0.9 Hz, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ ppm 26.70, 29.56, 29.84, 37.76, 41.08, 57.00, 61.69, 63.42, 120.80, 131.90, 141.07, 142.35, 166.18, 174.66; HRMS (ES) m/z: calcd for $C_{16}H_{23}N_3O_4SAs$ ($M+H^+$): 428.0625, found: 428.0598.

4.4. Synthesis of *N*-(4-[1,3,2]dithiarsolan-2-yl-phenyl)hexahydro-2-oxo-(3a*S*,4*S*,6a*R*)-1*H*-trieno[3,4-*d*]imidazole-4-pentamide (6)

To a stirred solution of **5** (152.5 mg, 0.3569 mmol) in MeOH (20 mL) at room temperature was added 1,2-ethanedithiol (31.5 μL, 0.3605 mmol). After 1 h, the suspension was cooled in ice and the solid was collected by filtration, washed with cold MeOH and dried under vacuum to afford 149.5 mg of **6**. White solid; 86% yield; mp 157–159 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.29–1.71 (m, 6H), 2.31 (t, J = 7.3 Hz, 2H), 2.57 (d, J = 12.3 Hz, 1H), 2.82 (dd, J = 12.6 Hz, J = 5.1 Hz, 1H), 3.07–3.22 (m, 3H), 3.29–3.41 (m, 2H), 4.10–4.17 (m, 1H), 4.27–4.34 (m, 1H), 6.36 (s, 1H), 6.43 (s, 1H), 7.55 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 9.98 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6)δ ppm 24.96, 27.98, 28.11, 36.12, 41.25 (2C), 55.28, 59.09, 60.93, 118.64 (2C), 131.18 (2C), 136.79, 140.12, 162.58, 171.27; HRMS (ES) m/z: calcd for $C_{18}H_{25}N_3O_2S_3As$ ($M+H^+$): 486.0325, found: 486.0332.

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