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Preparation of 3-^{125}I-Benzyl-(6R,7S)-7-Methoxy-3-Acetoxymethyl-3-Cephem-4-Carboxamide-1,1-Dioxide

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SUMMARY

The ^{125}I-labeled cephalosporin amide **1** was prepared via chloramine-T-mediated iodo-tin exchange of the tributyltin precursor **2**.

Key words: Apoptosis, ^{125}I, Cephalosporin, Caspase, ICE.

Introduction

Apoptosis has been shown to play a role in numerous degenerative diseases in which inappropriate cell death occurs.[1] Caspases (homologous enzymes within the Interleukin-1β Converting Enzyme [ICE] family) have been characterized as integral components of signal transduction cascades leading to apoptosis in several cellular models.[2] Thus, inhibition of Caspases, and hence apoptosis, can be a means by which one treats or prevents these degenerative diseases. The unlabeled cephalosporin **1** was discovered to be a time-dependent inhibitor of human recombinant ICE,[3] and was also demonstrated to be active in an HL-60 cell based model of apoptosis.[4] Further, the mechanism of apoptosis inhibition by **1** was shown to involve the irreversible labeling of protein in HL-60 cells.[3] Since there exists at least ten Caspases (most of which are ubiquitously expressed), there was the possibility that the cellular target(s) of **1** in HL-60 cells was not simply ICE itself. Therefore, radioaffinity label **I** was synthesized as a tool for identifying the relevant cellular target(s).

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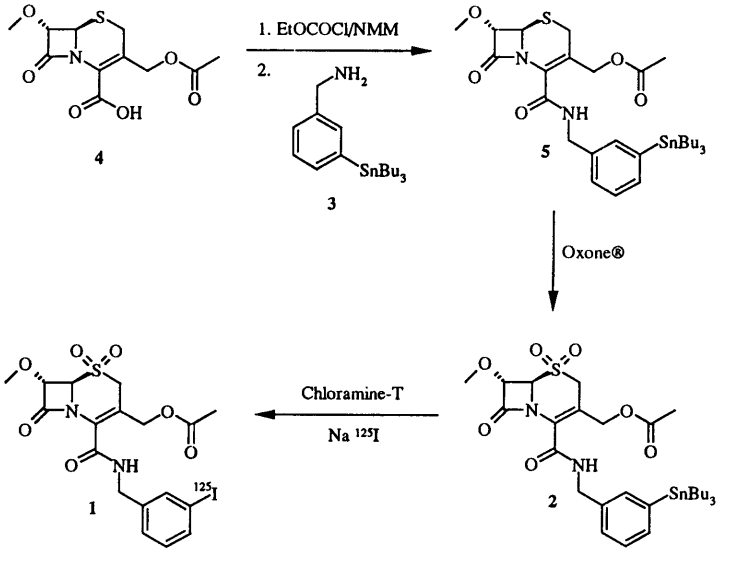
Results and Discussion

It was envisioned that radiolabeled **1** could be prepared from the tributyl tin precursor **2** via chloramine-T-mediated iodo-tin exchange. An initial attempt to prepare the tin intermediate via a palladium mediated exchange of unlabeled **1** with bis-tributyltin was unsuccessful. This reaction yielded a complex mixture, and no desired product could be isolated. An alternative procedure in which the preformed stannyl-substituted benzylamine is coupled to the cephalosporin nucleus was then investigated. This strategy provided a more convergent synthesis, and thus minimized chemistry on the sensitive cephalosporin nucleus. In addition, by choosing a route which did not involve unlabeled 3-iodobenzylamine, the possibility of contamination of the final product by unlabeled **1** was avoided.[5] 3-(Tri-*n*-butylstannyl)benzylamine (**3**) was prepared from 3-bromobenzylamine according to the reported literature procedure.[6] Activation of the carboxyl in cephalosporin **4**, followed by addition of the amine **3** afforded amide **5** in 60% yield. Oxidation with Oxone® afforded the sulfone **2** in 43% yield.



The radiolabeled cephalosporin amide **1** was obtained utilizing a radioiododestannylation procedure employing chloramine-T as oxidant.[7] Conversion of 2 to **1** was achieved in 64% radiochemical yield using 5 mCi of $Na^{125}I$ with chloramine-T in 3% acetic acid/ethanol.

Conclusion

Chloramine-T mediated radioiododestannylation of **2** afforded the cephalosporin amide **1** in 64% radiochemical yield and a high specific activity of 1100 Ci/mmol. To the best of our knowledge, this is the first reported example of performing the radioiododestannylation procedure on a cephalosporin nucleus.[8,9]

Experimental

General: All chemicals were obtained from the Aldrich Chemical Company unless otherwise noted. THF was distilled from sodium and benzophenone prior to use. Proton nuclear magnetic resonance spectra were obtained on a Bruker AM-400 instrument. Chemical shifts (δ) are referenced to residual solvent 7.26 ppm for chloroform.

3-(Tri-*n*-butyltin)benzyl-(6R, 7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxamide (**5**)

To a solution of 0.90 g (3.13 mmol) of cephalosporonic acid **4** in 40 mL of dry THF at -23 °C was added 0.70 mL (4.70 mmol) of N-methylmorpholine. After stirring 5 minutes, 0.45 mL (4.70 mmol) of ethyl chloroformate was added. The resulting solution was stirred for 15 minutes, and 1.86 g (4.70 mmol) of 3-(tri-*n*-butylstannyl)benzylamine was added in 10 mL of dry THF. The solution was allowed to warm to 0 °C over 1.5 h with stirring. Water was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a light yellow solid. Purification by flash chromatography (25-55% ethyl acetate/hexanes) afforded 1.25 g (60%) of **5** as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.31 (4H, m, Ar-H), 7.18 (1H, br t, NH), 4.84 (2H, AB q, J=13 Hz, OCH_2), 4.69 (1H, d, J=1.8 Hz, OCH), 4.58 (2H, m , $ArCH_2$), 4.52 (1H, d, J=1.8 Hz), 3.56 (3H, s, OCH_3), 3.41(2H, AB q, J=18 Hz, SCH_2), 2.04 (3H, s, $COCH_3$), 1.52-1.06 (18H, m, $(CH_2)_3CH_3$), 0.89 (9H, t, J=7.32 Hz, $(CH_2)_3CH_3$).

3-(Tri-*n*-butyltin)benzyl-(6R, 7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxamide-1,1-dioxide (**2**)

To 49 mg (0.074 mmol) of the sulfide **5** in 1.5 mL of CH_3CN at 0 °C was added 136 mg (0.221 mmol) of Oxone® in 1.5 mL of water. The mixture was warmed to room temperature and stirred for 2 days. CH_2Cl_2 was added to the reaction, and the mixture was washed with 20% $Na_2S_2O_5$, sat. $NaHCO_3$ and water. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give an

oil. Purification by flash chromatography (50% ethyl acetate/hexanes) afforded 22 mg (43%) of **2** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.30 (4H, m, Ar-H), 7.37 (1H, br t, NH), 5.15 (1H, d, J=1.8 Hz, OCH), 4.78 (2H, AB q, J=14 Hz, OCH_2), 4.72 (1H, d, J=1.3 Hz), 4.56 (2H, m , $ArCH_2$), 3.81 (2H, AB q, J=18 Hz, SCH_2), 3.56 (3H, s, OCH_3), 2.06 (3H, s, $COCH_3$), 1.53-1.05 (18H, m, $(CH_2)_3CH_3$), 0.89 (9H, t, J=7.27 Hz, $(CH_2)_3CH_3$).

3-^{125}I-benzyl-(6R, 7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxamide-1,1-dioxide (**1**)

To a solution containing 100 ug (143 nmol) of stannane **2** in 100 uL of 3% acetic acid in ethanol was added 5.0 mCi (2.5 nmol) of sodium iodide-^{125}I (DuPont/NEN) in 40 uL of 0.1 N NaOH and 2 uL (8.8 nmol) of a chloramine-T solution in water (1ug/uL). The reaction mixture was kept at room temperature in the dark for 50 minutes. The reaction was then concentrated under nitrogen, dissolved in 50 uL of mobile phase and purified (one injection *via* a Rheodyne 7125 injector onto a Baker SiO_2, 5um, 4.6 mm x 250 mm HPLC column eluted with 40:60 (v/v) ethyl acetate/hexane at a flow rate of 1.5 mL/min, UV detection @ 260 nm (Waters 481 detector), with radioactivity detected by a flow-through NaI(Tl) crystal scintillation detector comprised of Ludlum components). The desired radioactive product (retention time = 23.4 min.vs. 10.2 min. for stannane **2**) was collected using a Gilson automatic fraction collector. The labeled product (3.2 mCi total, 64% radiochemical yield) was analyzed by normal phase HPLC and found to have a radiochemical purity of 99.5%, with specific activity of 1100 Ci/mmol (derived from mass concentration using a standard HPLC mass/UV response curve and radioactive concentration). .It was found that radiolabeled **1** was unstable when stored in DMSO (RCP fell rapidly to 21-57% when stored at -78 °C), but exhibited much greater stability when stored in 40:60 (v/v) ethyl acetate/hexane or ethanol at -78 °C (>99% RCP).

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5. Contamination with unlabeled **1** would result in dilution of the specific activity of radiolabel **1**.

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8. All prior examples of preparations of radiolabeled cephalosporins involved the coupling of a labeled reagent with the cephalosporin nucleus. See: Rojo, F., Ayala, J. A., De La Rosa, E. J., De Pedro, M. A., Aran, V., Berenguer, J., Vazquez, D.- J. Antibiot. **37**: 389 (1984). and Schwarz, U., Seeger, K., Wengenmayer, F., Strecker, H.- FEMS Microbiol. Lett. **10**: 107 (1981).

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