Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 7, pp. 779-784, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00106-0

One-Step Synthesis of Radioiodinated Biotin Derivatives

Catherine F. Foulon, S. James Adelstein and Amin I. Kassis*

Harvard Medical School, Department of Radiology

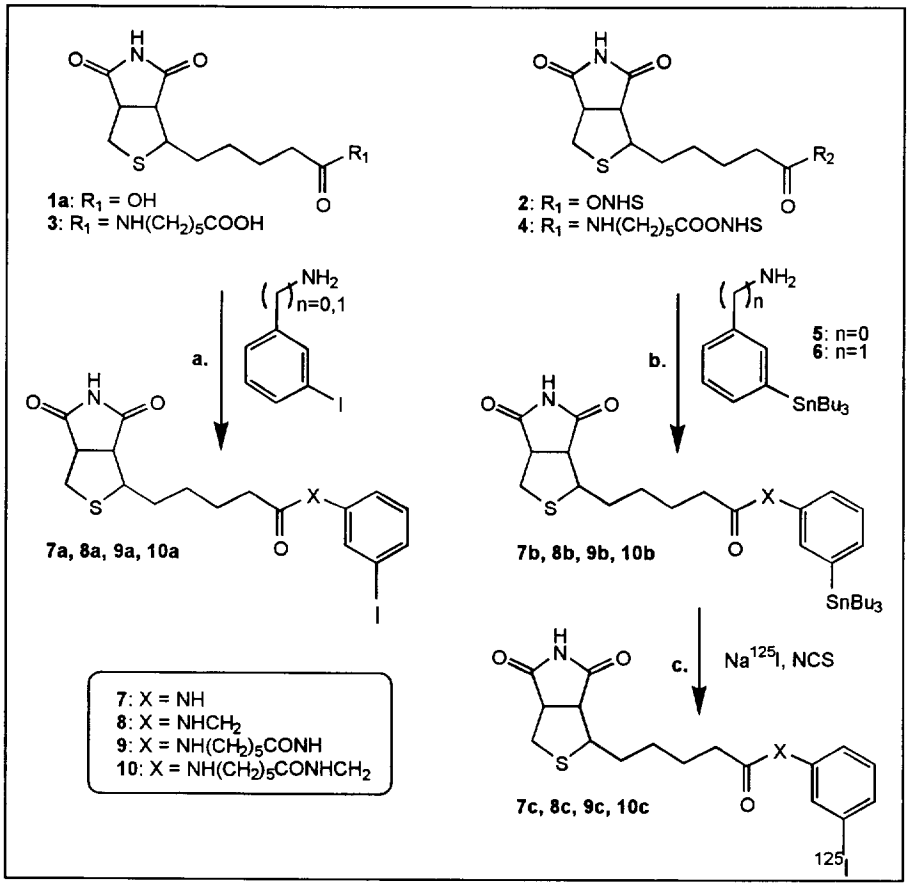
50 Binney Street, Boston, MA 02115

Abstract. A direct method of radioiodination of biotin derivatives is presented whereby radiolabeled sodium iodide is exchanged with a tributylstannyl group under oxidative conditions. The in vitro binding of each radioiodinated biotin derivative to streptavidin is saturable and three of the compounds exhibit a strong affinity for streptavidin. All the biotin-streptavidin complexes are rapidly catabolized in human serum.

Biotin (**1a**), a 244 Da vitamin, exhibits a very high affinity ($K_a = 10^{15}$ M^{-1}) for both avidin and streptavidin, 65 kDa proteins,[1] and the biotin-avidin duo is now an established technology for bridging molecules that have no affinity for each other.[2] Studies and applications with the biotin-avidin complex would greatly benefit from the availability of radiolabeled biotin derivatives, especially when using no-carrier-added radionuclides such as ^{123}I and ^{125}I. Direct radioiodination of biotin has so far been avoided because of the sensitivity of the vitamin to oxidizing agents. Radioiodinated biotin derivatives have been prepared either by a two-step synthesis, in which the prosthetic group is first radiolabeled and then coupled to activated biotin,[3] or by dediazotization.[4] We present here a series of biotin derivatives as a model for the preparation of such radioiodinated ligands by direct demetallation of the respective tributylstannyl precursor.

Biotinylated intermediates N-hydroxysuccinimidobiotin (**2**), biotinyl-γ-caproate (**3**), and γ-caproylamidobiotin-N-hydroxysuccinimide ester (**4**), as well as the tributylstannyl prosthetic groups, 3-tributylstannyl aniline (**5**) and 3-tributylstannyl benzylamine (**6**), were prepared as described in the literature (Scheme I).[5] The nonradioactive iodinated derivatives, biotinyl-3-iodoanilide (**7a**), biotinyl-3-iodobenzylamide (**8a**), biotinyl-γ-caproyl-3-iodoanilide (**9a**) and biotinyl-γ-caproyl-3-iodobenzyl-amide (**10a**), were obtained by first reacting 0.56 mmol biotin derivative, **1a** or **3**, dissolved in 3 mL DMF (it may be necessary to warm the solution up to 80 °C) with 1 mmol isobutylchloroformate and 1 mmol triethylamine at 0 °C for 10 min, followed by the action at room temperature for 15 min of 0.82 mmol of either 3-iodoaniline to obtain **7a** and **9a** or 3-iodobenzylamine to obtain **8a** and **10a**. The solvent was evaporated and the product was purified by preparative TLC (silica gel, 2000 μm, dichloromethane:methanol, 90:10; R_f: **7a** = 0.73, **8a** = 0.61, **9a** = 0.51, **10a** = 0.48). The desired band of silica gel was collected and stirred in dichloromethane:methanol, 90:10, for 20 min,

and then the solvent was filtered and removed by rotary evaporation. The white solid, thus obtained, was recrystallized in methanol to afford the desired product as white crystals, with an average yield of 70%.[6] The retention time of each iodinated derivative was assessed by HPLC (C_{18} column, methanol:water, 65:35, 1 mL/min) so that the identity of the radioiodinated derivative could be ascertained at a later time: **7a** and **8a** eluted at 9 min; **9a** and **10a** eluted at 9.5 min. Attempts to synthesize **7a-10a** by reacting 3-iodoaniline or 3-iodobenzylamine with either **2** or **4** were not successful. Since the presence of hydrochloric acid would cause destannylation, the tributylstannyl derivatives (**7b**, **8b**, **9b** or **10b**) were not produced by the above route. Each metallic precursor was



Scheme I. Preparation of cold iodinated (**a**), tributylstannyl (**b**) and radioiodinated (**c**) derivatives of **7**, **8**, **9** and **10**

was obtained by reacting 0.60 mmol of the appropriate N-succinimidobiotin derivative, **2** or **4**, with 0.64 mmol of either 3-tributylstannyl aniline (**5**) to obtain **7b** or **9b** or 3-tributylstannyl benzylamine (**6**) to obtain **8b** or **10b**. Each reaction was performed in 3 mL DMF, pH adjusted to above 8 with 0.1 M sodium bicarbonate solution, and the mixture was stirred at room temperature for 18 h. The solvent was then evaporated and the product was purified by preparative TLC (silica gel, 2000 μm, dichloromethane:methanol, 90:10; R_f: **7b** = 0.78, **8b** = 0.68, **9b** = 0.56, **10b** = 0.55). Following chromatography, the silica gel containing the desired product was scraped and stirred in dichlorometane:methanol, 90:10, for 20 min; the solvent was filtered; and the product was concentrated using rotary evaporation to afford a white powder. Each precursor was recrystallized in ethyl acetate:hexane, 20:80, to give white crystals, with an average yield of 60%.[7] When the purified products were injected into HPLC (system described above), none were eluted. To do so, it was necessary to wash the column with 100% methanol. All precursors were aliquoted (100 μg in 50 μL methanol) and kept in the freezer until radiolabeling.

The radioiodinated derivative (**7c**, **8c**, **9c** or **10c**) was prepared by addition of $Na^{125}I$ (Amersham Corporation, Arlington Heights, IL; 5-10 μL, 0.5-1 mCi) to each of the tributylstannyl precursors (**7b**, **8b**, **9b** or **10b**, 100 μg in 50 μL methanol) along with N-chlorosuccinimide (20 μL, 1mg/mL methanol) as the oxidative agent. The vial was vortexed for a maximum of 10 sec and the reaction was immediately quenched by adding 200 μL saturated aqueous sodium metabisulfite solution and 200 μL water. It is imperative to quench the reaction rapidly to prevent formation of the sulfoxide by-products, which do not exhibit any affinity for streptavidin. The sample was eluted through a tC_{18} cartridge (Millipore Corporation, Bedford, MA) with 1 mL methanol:water, 70:30, a system in which the tributylstannyl precursor did not elute. The filtrate was then diluted in 5 mL water and placed on a second tC18 cartridge in order to concentrate the sample. The desired product was eluted in methanol (300 μL) which was evaporated under a stream of nitrogen. The sample was then reconstituted in dichloromethane (50 μL) and loaded on a Baker-flex silica gel IB2-F plate (2.5 x 7.5 cm, 250 μm, J. T. Baker, Incorporated, Phillipsburg, NJ) which was eluted with dichloromethane:methanol, 90:10. The band matching the R_f of the cold standard was scraped off and mixed with dichloromethane. After centrifugation, the supernatant was removed, and the solvent was evaporated under a stream of nitrogen. The plate purification was found necessary to remove any biotinylated by-products. The total preparation time was 1 hr with an average radiochemical yield of 45% and radiochemical purity of 95%. For quality control, each radioiodinated derivative was co-injected into HPLC with the respective cold standard. The derivatives were stable when kept

in methanol or water at room temperature for at least two weeks. In the absence of treatment with sodium metabisulfite, the sulfoxide by-product was eluted at 6 min on HPLC. Once oxidized, it was not possible to recover the reduced form of biotin; addition of an excess of sodium metabisulfite was fruitless. We concluded that the kinetics of iododestannylation are faster than those of sulfur oxidation.

The in vitro binding of the radioiodinated biotin derivatives to streptavidin was assessed as follows. A constant amount (18 nM, 4 μCi in 100 μL distilled water) of each radioligand (**7c**, **8c**, **9c** or **10c**) was incubated with an increasing amount of streptavidin (Pierce, Rockford, IL; 0, 20, 200, 1000, 2000 and 3000 nM in 100 μL distilled water). The samples were placed on an orbital shaker for 18 h and 3 μL were then spotted on ITLC (silica gel; Gelman Sciences Incorporated, Ann Arbor, MI). The plates were eluted with 0.15 M NaCl:ethanol, 95:5. The radioiodinated biotin-straptavidin complex stayed at the baseline whereas the free tracer moved upward. The plate was cut horizontally 1.5 cm above the origin; the radioactive contents of the two parts were quantitated; and the percentage of biotin-streptavidin complex (% biotin bound) was calculated as the ratio (activity at the baseline)/(activity at the baseline + activity on the upper part of the plate). As a reference, tritiated biotin (**1b**, Du Pont NEN Research Products, Boston, MA; 18 nM in 100 μL distilled water) was tested under the same conditions. The curve % radiolabeled biotin bound versus streptavidin concentration was plotted for each radiotracer (Figure 1). The binding was found saturable and **7c**, **8c** and **9c** exhibited a strong affinity for streptavidin that was similar to that of **1b**. The binding was stable with time (up to three weeks at room temperature).

Finally, each radioiodinated ligand (4 μCi in 100 μL distilled water) was incubated with 3 mM streptavidin solution (100 μL) for 18 h. The formation of each complex was ascertained by ITLC as described above. The radioiodinated biotin-streptavidin conjugate (5 μL) was then incubated with human serum (50 μL) at 37 °C and its stability was monitored by ITLC at various time intervals (Figure 2). All complexes were rapidly catabolized (in less than 5 min), a circumstance that limits their use in vivo.

Summary

We report here a convenient preparation for radioiodinated biotin derivatives by direct labeling of their tributylstannyl precursors under oxidative conditions. Prompt treatment with sodium metabisulfite is necessary to prevent the oxidation of the sulfur. Although **7c**, **8c**, and **9c** display a very high affinity for streptavidin and stability in aqueous solution, their poor stability in

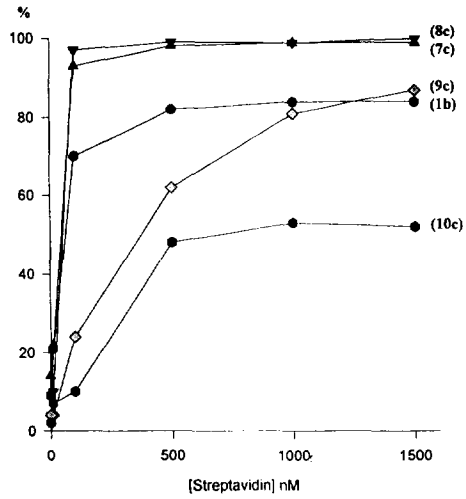


Figure 1. Saturation curve of percent radiolabeled biotin bound to streptavidin.

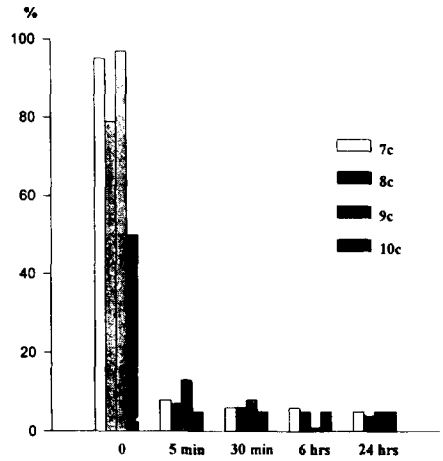


Figure 2. In vitro stability of radioiodinated biotin-streptavidin complexes in human serum.

human serum is an obstacle to use in vivo. This series was used as a model for the synthesis of radioiodinated derivatives of biotin by direct destannylation, and other derivatives designed to have improved pharmacokinetics are under investigation.

References

1. a. Green, N. M. *Adv. Protein Chem.* **1975,** *29*, 85. b. Chaiet, L.; Wolf, F. J. *Arch. Biochem. Biophys.* **1964,** *106*, 1.

2. a. Wilchek, M.; Bayer, E. A. *Methods Enzymol.* **1990,** *184*, 5. b. Savage, M. D.; Mattson, G.; Desai, S.; Nielander, G. W.; Morgensen, S.; Conklin, E. G. *Avidin-Biotin Chemistry: A Handbook;* Pierce Chemical Company: Rockford; 1992.

3. a. Khawli, L. A.; Kassis, A. I. *Nucl. Med. Biol.* **1992,** *19*, 297. b. Garlick, R. K.; Giese, R. W. *J. Biol. Chem.* **1988,** *263*, 210. c. Livianou, E.; Evangelatos, G. P.; Ithakissios, D. S. *J. Nucl. Med.* **1987,** *28*, 1430.

4. Kortylewicz, Z. P.; Baranowska-Kortylewicz, J.; Adelstein, S. J.; Carmel, A. D.; Kassis, A. I. *J. Labelled Compd. Radiopharm.* **1994,** *34*, 1129.

5. All chemicals, unless otherwise indicated, were provided by Aldrich Chemical Company, Milwaukee, WI. a. For product **2**: Jasiewicz, M. L.; Schoenberg, D. R.; Mueller, G. C. *Exp. Cell Res.* **1976,** *100*, 213. b. For products **3** and **4**: Costello, S. M.; Felix, R. T.; Giese, R. W. *Clin. Chem.* **1979,** *25*, 1572. c. For product **5**: see reference 3a. d. For product **6**: Zalutsky, M. R.; Narula, A. S. *Appl. Radiat. Isot.* **1987,** *38*, 1051.

6. Elemental analysis: C, H, N. **7a**, Calcd: C = 43.15, H = 4.53, N = 9.44; Found: C = 42.66, H = 4.61, N = 9.62. **8a**, Calcd: C = 44.45, H = 4.79, N = 9.15; Found: C = 44.16, H = 4.94, N = 9.00. **9a**, Calcd: C = 47.55, H = 5.89, N = 10.56; Found: C = 48.47, H = 6.23, N = 10.49. **10a**, Calcd: C = 48.25, H = 5.81, N = 9.79; Found: C = 46.64, H = 5.87, N = 9.45.

7. ^{1}H-NMR (DMSO). **7b**: 9.87 (s, 1H, NHCO), 8.16 (dd, 1H, ArH2), 7.58-6.96 (m, 3H, ArH), 6.62 (s, 1H, NHCO), 5.61 (s, 1H, NHCO), 4.28 (m, 2H, 2CHN), 3.12 (m, 1H, SCH), 2.72 (m, 2H, SCH_2), 2.20 (m, 2H, CH_2CO), 1.82-0.85 (m, 33H, 3nBu+$(CH_2)_3$). Elemental analysis: C, H, N. **7b**, Calcd: C = 55.27, H = 7.79, N = 6.91; Found: C = 51.38, H = 7.08, N = 7.10. **8b**, Calcd: C = 55.96, H = 7.93, N = 6.75; Found: C = 55.83, H = 7.95, N = 6.79. **9b**, Calcd: C = 56.59, H = 8.10, N = 7.76; Found: C = 49.64, H = 6.96, N = 10.10. **10b**, Calcd: C = 57.15, H = 8.22, N = 7.62; Found: C = 56.06, H = 7.89, N = 7.64.

(*Received in USA* 31 *October* 1995; *accepted* 21 *February* 1996)