Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1281–1284

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ESTRADIOL-MIMETIC PROBES. PREPARATION OF 17α-(6-AMINOHEXYNYL)ESTRADIOL BIOTIN, FLUORESCEIN AND ACRIDINIUM CONJUGATES

Maciej Adamczyk,* Yon-Yih Chen, Jeffrey A. Moore, Phillip G. Mattingly

Department of Chemistry, Diagnostics Division, Abbott Laboratories, 100 Abbott Park Road, Dept. 09NM, Bldg. AP-20, Abbott Park, IL 60064-3500, U. S. A.

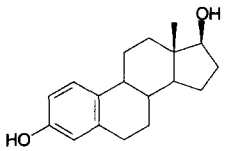
Received 3 February 1998; accepted 26 March 1998

Abstract: 3-*O*-*tert*-Butyldimethylsilyl-17α-(6-mesyloxyhexynyl)estradiol was converted to the azide in 60–70% yield with NaN_3/DMPU, then reduced to the corresponding amine (>95% yield). Acylation with the *N*-hydroxysuccinimide esters of biotin, 5-carboxyfluorescein and 10-(3-sulfopropyl)-*N*-tosyl-*N*-(3-carboxypropyl)acridinium-9-carboxamide gave the title conjugates. The K_Ds of the tracers with an estradiol antibody ranged from 97–197 nM.

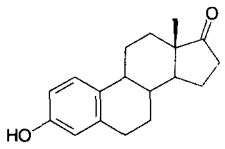
Estradiol (E_2, **1**) is a hormone which is necessary for development of secondary sexual characteristics (e.g., hair, body shape, etc.) and function of the reproductive system in females. It also plays an important role in the etiology of estrogen-dependent tumors and in other endocrinological disorders.[1–3] As such, many labeled E_2 derivatives have been reported for probing estrogen binding proteins (i.e., estrogen receptors[4] or antibodies) and for use in commercially important immunoassays for E_2.[5–9] The most straightforward way of preparing a labeled probe of estradiol is conjugation through the C3[10–12] or C17 hydroxyl groups,[11,13,14] however, this approach blocks those critical binding determinants, rendering them less than perfect mimics of estradiol during binding to natural receptors or antibodies. An alternative strategy is to introduce the conjugate at a new functional group, remote from these determinants which minimally perturbs the native structure of estradiol. To this end, reactive functional groups have been introduced at the C6[11,15–20] and C7[21–25] positions of estradiol. The C6 derivatives all issued from 6-oxoestradiol for which there was no efficient synthesis until recently.[26] For all but one,[21] the syntheses of the C7 derivatives were complicated by using U. S. Drug Enforcement Agency (DEA) controlled starting materials. Recently, several groups have made use of the facile stereoselective addition of alkynyl lithium compounds to the 17-keto group of estrone (**2**) to generate 17α-alkynyl estradiol derivatives bearing pendant hydroxy,[27–29] bromoacetoxy[28] or sulfhydryl groups.[29] This approach allowed for the easy introduction of linking groups of variable length. Lacking though, was the preparation of the corresponding amino derivatives. We now report the preparation of 17α-(6-aminohexynyl)estradiol (**5**) and its subsequent conversion to non-radioactive probes containing the desirable

0960-894X/98/$19.00
PII: S0960-894X(98)00225-X

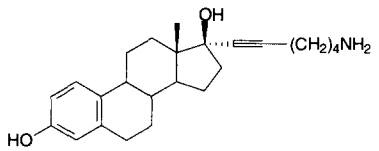
labels, biotin and fluorescein, along with a newly introduced chemiluminescent acridinium label. Preliminary evaluation of their binding properties with an anti-E_2 monoclonal antibody is also reported.



1



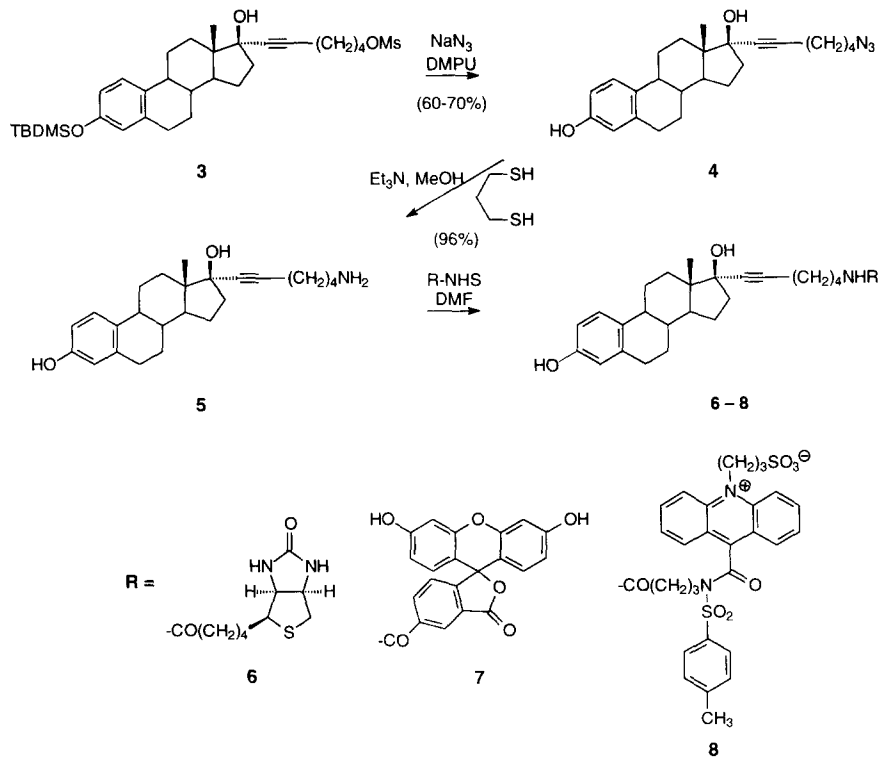
2



5

The recently reported mesylate **3**,[29] prepared from the corresponding alcohol that resulted from the addition of dilithio-5-hexyn-1-ol to 3-*O*-*tert*-butyldimethylsilyl protected estrone, was reacted with an excess of sodium azide in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU)[30] over the course of 3–5 days at ambient temperature to give the azide **4**[31] (60–70%) with concomitant removal of the TBDMS protecting group. Selective reduction of the azide[32] to amine **5** (96% yield after silica gel chromatography) proceeded smoothly, but slowly, over the course of 5 days in the presence of a 12-fold excess of 1,3-propanethiol and triethylamine. 17α-(6-Aminohexynyl)estradiol (**5**) was conjugated to biotin,[33] 5-carboxyfluorescein,[34] and 10-(3-sulfopropyl)-*N*-tosyl-*N*-(3-carboxypropyl)-acridinium-9-carboxamide[35,36] via the corresponding *N*-hydroxysuccinimide (NHS) esters in DMF to produce the labeled estradiol probes **6–8** (74, 73, and 26% yield, respectively, after purification by preparative reversed-phase HPLC.)

To assess their suitability as estradiol-mimetic probes in an immunoassay format, a preliminary evaluation of the solution binding affinities of compounds **6–8** for an anti-estradiol-6-carboxymethyloxime mAb was carried out. Binding studies were conducted using a competitive equilibrium assay on a BIAcore 2000 instrument.[37–39] 7α-(3′-Carboxypropyl)estradiol[21] containing an ethylenediamine linker was immobilized on a CM-5 sensor chip and served as the anti-estradiol-6-CMO mAb biosensor. The apparent affinity of anti-estradiol-6-CMO mAb for this surface, determined in a direct equilibrium binding format, was 23 ± 5.9 nM. Solution binding affinities of compounds **6–8** for the anti-estradiol-6-CMO mAb were subsequently determined from equilibrated mixtures of the tracer and antibody which were injected over the calibrated biosensor matrix. After correction for bivalency of the mAb,[40,41] equilibrium dissociation constants (K_D) of 141.1 ± 18.8 nM, 97.3 ± 9.9 nM, and 197.5 ± 32.2 were obtained for compounds **6–8**, respectively. The introduction of the 17α-alkynyl-label resulted in a 40 fold decrease in K_D in comparison to unlabeled estradiol. This binding affinity is, however, in a range suitable for competitive immunoassay. The type of label incorporated into the tracer had a noticeable affect on its binding efficiency with the antibody. We have observed this phenomena with other tracer/antibody combinations, but any trends are highly antibody dependent, and not easily elucidated. One could speculate, though, that the differences indicate a secondary binding interaction between the antibody and one of the several possible determinants on the label. Such factors as the *pi*-system of the fluorescein or acridinium groups, the ionization state of the label, or its ability to form hydrogen bonds, may play a part.



Scheme

REFERENCES AND NOTES

1. Lippman, M. E.; Allegra, J. C. *N. Engl. J. Med.* **1978,** *299*, 930.
2. Dence, J. B. *Steroids and Peptides*; Wiley: New York, 1980.
3. Top, S.; El Hafa, H.; Vessières, A.; Quivy, J.; Vaissermann, J.; Hughes, D. W.; McGlinchey, M. J.; Mornon, J.-P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc.* **1995,** *117*, 8373.
4. Anstead, G. M.; Carlson, K. E. *Steroids* **1997,** *62*, 268.
5. Chan, D. W.; Perlstein, M. T. *Immunoassay. A Practical Guide*; Academic: New York, 1987.
6. Alberton, B. D.; Haseltine, F. P. *Non-radiometric Assays. Technology and Application in Polypeptide and Steroid Hormone Detection*; A. R. Liss: New York, 1988.
7. Gosling, J. P. *Clin. Chem.* **1990,** *36*, 1408.
8. Wheeler, M. J. In *The Immunoassay Handbook;* Wild, D. Ed.; Stockton: New York, 1994; pp 366–378.
9. Bouma, S.; Worobec, S.; Baker, A.; Dubler, R.; Frias, E.; Ginsburg, S.; Hehmann, M.; Ishman, S.; Gardiner, M.; Hsu, S.; Pacenti, D.; Pascucci, T.; Patel, C.; Piechura, J.; Schueller, M.; Sheu, M.; Wendland, D.; Wang, P. *Clin. Chem.* **1997,** *43*, 295.
10. Sato, H.; Mochizuki, H.; Tomita, Y.; Izako, T.; Sato, N.; Kanamori, T. *J. Biolumin. Chemilumin.* **1996,** *11*, 23.
11. De Boever, J. G.; Kohen, F.; Bosmans, E. *Anal. Chim. Acta* **1993,** *275*, 81.
12. Patel, A.; Woodhead, J. S.; Campbell, A. K.; Hart, R. C.; McCapra, F. *Serono Symp. Publ. Raven Press* **1982,** *1*, 181.
13. Barrows, G. H.; Stroupe, S. B.; Riehm, J. D. *Am. J. Clin. Path.* **1980,** *73*, 330.
14. Daxenbichler, G.; Grill, H. J.; Domanid, R.; Moser, E.; Dapunt, O. *J. Steroid Biochem.* **1980,** *13*, 489.
15. Adamczyk, M.; Mattingly, P. G.; Reddy, R. E. *Steroids* **1997,** *62*, 462.
16. Mares, A.; DeBoever, J.; Stans, G.; Bosmans, E.; Kohen, F. *J. Immunol. Methods* **1995,** *183*, 211.

17. Luppa, P.; Birkmayer, C.; Hauptmann, H. *Bioconjugate Chem.* **1994,** *5*, 167.
18. Tiefenauer, L. X.; Andres, R. Y. *J. Steroid Biochem.* **1990,** *35*, 633.
19. Bodmer, D. M.; Tiefenauer, L. X.; Andres, R. Y. *J. Steroid Biochem.* **1989,** *33*, 1161.
20. Dandliker, W. B.; Hicks, A. N.; Levison, S. A.; Brawn, R. J. *Biochem. Biophys. Res. Commun.* **1977,** *74*, 538.
21. Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Steroids* **1997,** *62*, 771.
22. French, A. N.; Wilson, S. R.; Welch, M. J.; Katzenellenbogen, J. A. *Steroids* **1993,** *58*, 157.
23. Redeuilh, G.; Secco, C.; Baulieu, E. E. *Methods Enzymol.* **1990,** *184*, 292.
24. Muhelenbruch, B.; Kirmeier, F.; Roth, H. J. *Arch. Pharm. (Weinheim)* **1986,** *319*, 196.
25. Bucourt, R.; Vignau, M.; Torelli, V.; Richard-Foy, H.; Geynet, C.; Secco-Millet, C.; Redeuilh, G.; Baulieu, E. E. *J. Biol. Chem.* **1978,** *253*, 8221.
26. Brevet, J.; Fournet, G.; Goré, J. *Synthetic Commun.* **1996,** *26*, 4185.
27. Salman, M.; Reddy, B. R.; Ray, S.; Stotter, P. L.; Chamness, G. C. *J. Steroid Biochem.* **1986,** *24*, 539.
28. El Garrouj, D.; Aumelas, A.; Borgna, J.-L. *J. Med. Chem.* **1993,** *36*, 2973.
29. Wüst, F.; Spies, H.; Johannsen, B. *Tetrahedron Lett.* **1997,** *38*, 2931.
30. Davis, A. P.; Dresen, S.; Lawless, L. J. *Tetrahedron Lett.* **1997,** *38*, 4305.
31. Analytical Data: Compound **4** (oil): ^{1}H NMR (δ, $CDCl_3$) 7.17 (1 H, d, J = 8.5 Hz), 6.64 (1 H, dd, J = 1.5, 8.5 Hz), 6.57 (1 H, d, J = 1.5. Hz), 5.09 (1 H, s), 3.31 (2 H, t, J = 6.6 Hz), 2.82 (2 H, m), 2.31 (2 H, t, J = 6.9 Hz), 2.1–2.4 (3 H, m), 1.30–2.05 (15 H, m), 0.87 (3 H, s); ^{13}C NMR (δ, $CDCl_3$) 153.51, 138.33, 132.60, 126.61, 115.29, 112.73, 85.45, 84.35, 80.09, 50.85, 49.45, 47.10, 43.58, 39.32, 39.02, 32.82, 29.53, 27.87, 27.10, 26.32, 25.74, 22.66, 18.28, 12.69; ν_{max} (film from $CHCl_3$) 2094 cm^{-1} (N_3); ESI-MS *m/z* 394 $(M + H)^+$; HPLC [3.9 × 300 mm μBondapak C18, CH_3CN:water:CH_3CO_2H (50:50:0.1), 1 mL/min, 254 nm] 8.54 min. Compound **5**: ^{1}H NMR (δ, CD_3OD) 7.08 (1 H, d, J = 8.4 Hz), 6.54 (1 H, dd, J = 1.5, 8.4 Hz), 6.47 (1 H, d, J = 1.5. Hz), 3.30 (2 H, m), 2.95 (2 H, m), 2.75–2.79 (2 H, m), 2.33 (2 H, t, J = 6.2 Hz), 1.30–2.05 (14 H, m), 0.85 (3 H, s); ^{13}C NMR (δ, $CDCl_3$) 154.13, 136.95, 130.58, 125.37, 114.18, 111.87, 83.96, 83.48, 78.67, 48.84, 46.37, 43.19, 39.10, 38.24, 37.98, 32.24, 28.69, 26.63, 25.77, 25.68, 24.74, 21.68, 16.95, 11.40; ESI-MS *m/z* 369 $(M + H)^+$; HPLC [3.9 × 300 mm μBondapak C18, CH_3CN:50 mM aq NH_4OAc (50:50), 1 mL/min, 254 nm] 7.37 min, 97%. Compound **6**: lyophilized solid; ^{1}H NMR (δ, CD_3OD) 7.09 (1 H, d, J = 9 Hz), 6.54 (1 H, dd, J = 9 and 3 Hz), 6.47 (1 H, d, J = 3 Hz), 4.47 (1 H, dd, J = 4 and 8 Hz), 4.26 (1 H, dd, J = 4 and 8 Hz), 3.19 (3 H, m), 2.91 (1 H, dd, J = 5 and 12 Hz), 2.76 (2 H, m), 2.69 (1 H, d, J = 12 Hz), 2.29 (2 H, t, J = 7 Hz), 2.12 (2 H, t, J = 8 Hz), 2.40–1.20 (23 H, m), 0.85 (3 H, s). ESI-MS *m/z* 595 $(M + H)^+$; HPLC [3.9 × 300 mm μBondapak C18, CH_3CN:0.05% aq TFA (40:60), 1 mL/min, 220 nm] 8.8 min, 95%. Compound **7**: lyophilized solid; ^{1}H NMR (δ, CD_3OD) 8.42 (1 H, d, J = 1 Hz), 8.08 (1 H, dd, J = 2 and 8 Hz), 7.17 (1 H, d, J = 8 Hz), 7.04 (1 H, d, J = 9 Hz), 6.80–6.58 (6 H, m), 6.46 (1 H, dd, J = 3 and 8 Hz), 6.39 (1 H, d, J = 3 Hz), 3.46 (2H, t, J = 7 Hz), 2.68 (2 H, m), 2.35 (2 H, t, J = 7 Hz), 2.40–1.20 (17 H, m), 0.84 (3 H, s). ESI-MS *m/z* 727 $(M + H)^+$; HPLC [3.9 × 300 mm μBondapak C18, CH_3CN:0.05% aq TFA (40:60), 1 mL/min, 254 nm] 17.15 min, 99%. Compound **8**: lyophilized solid; ^{1}H NMR (δ, CD_3OD) 8.91 (2 H, d, J = 9 Hz), 8.44 (2 H, t, J = 8 Hz), 8.22–6.80 (9 H, m), 6.41 (2 H, m), 5.72 (2 H, m), 4.19 and 3.44 (2 H, m, rotamers), 3.24 (2 H, t, J = 7 Hz), 2.80–1.10 (29 H, m), 0.82 (3 H, s). ESI-MS *m/z* 935 $(M + H)^+$; HPLC [3.9 × 300 mm μBondapak C18, CH_3CN:0.05% aq TFA (40:60), 1 mL/min, 254 nm] 15.1 min, 97%.
32. Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1976**, 3633.
33. Wilchek, M.; Bayer, E. A. *Methods Enzymol.* **1990,** *184*, 123.
34. Adamczyk, M.; Fishpaugh, J.; Heuser, K. *Bioconjugate Chem.* **1997,** *8*, 253.
35. Mattingly, P. G.; Bennett, L. U. S. Patent 1995, 5 468 646; www.patents.ibm.com.
36. Mattingly, P. G. *J. Biolumin. Chemilumin.* **1991,** *6*, 107.
37. Edwards, P. R.; Leatherbarrow, R. L. *Anal. Biochem.* **1997,** *246*, 1.
38. Morelock, M. M.; Ingraham, R. H.; Betageri, R.; Jakes, S. *J. Med. Chem.* **1995,** *38*, 1309.
39. Schuck, P. *Ann. Rev. Biophys. Biomol. Struct.* **1997,** *26*, 541.
40. Nieba, L.; Krebber, A.; Plückthun, A. *Anal. Biochem.* **1996,** *234*, 155.
41. Karlsson, R. *Anal. Biochem.* **1994,** *221*, 142.