Novel polyethylene glycol derivatives of melatonin and serotonin. Ligands for conjugation to fluorescent cadmium selenide/zinc sulfide core shell nanocrystals

J. Chem. Research (S), 2002, 203–204
J. Chem. Research (M), 2002, 0527–0539

Ian D. Tomlinson, Tadd Kippeny, Laura Swafford, Nasir H. Siddiqui and Sandra J. Rosenthal*

The Department of Chemistry, Vanderbilt University, Station B 351822 Nashville, Tennessee, 37235-1822, USA

This paper describes the synthesis and characterisation of derivatives of melatonin and serotonin that may be attached to highly fluorescent cadmium selenide/zinc sulfide core shells nanocrystals for use in biological assays and fluorescence imaging applications.

Keywords: serotonin, melatonin, cadmium selenide/zinc sulfide core shells, nanocrystal

The attachment of biologically active ligands to cadmium selenide nanocrystals coated with a zinc sulfide shell (core shells) is a new method of producing novel fluorescent sensors. Several groups have reported attaching proteins and antibodies to nanocrystals and core shells.[1-4] We have synthesised derivatives of melatonin and serotonin which may be attached to cadmium selenide/zinc sulfide core shell nanocrystals. Such ligand-conjugated nanocrystals can be used to image the localisation and distribution of membrane bound receptors, transporter proteins and ion channels in cell cultures.

Polyethylene glycol linker arms were attached to 5-hydroxytryptamine derivatives. A polyethylene glycol linker arm was used because polyethylene glycols increase the water solubility of the core shell conjugate and reduce steric interactions between the nanocrystal and the receptor.

In order to attach the linker arm to the 5-hydroxyindole derivative, it was necessary to synthesise a polyethylene glycol derivative that consisted of a protected thiol at one end of the chain and a leaving group at the other end of the chain. This chain may be attached to alcohols via a neucleophilic displacement of the leaving group. We chose the *para*-methoxybenzyl[7] protecting group to protect the thiol. This may be removed under a variety of conditions using silver or mercury salts or acid hydrolysis. Other protecting groups were considered[5,6] but rejected as they may be unstable under nucleophilic conditions or their methods of removal may not have been compatable with our ligands.

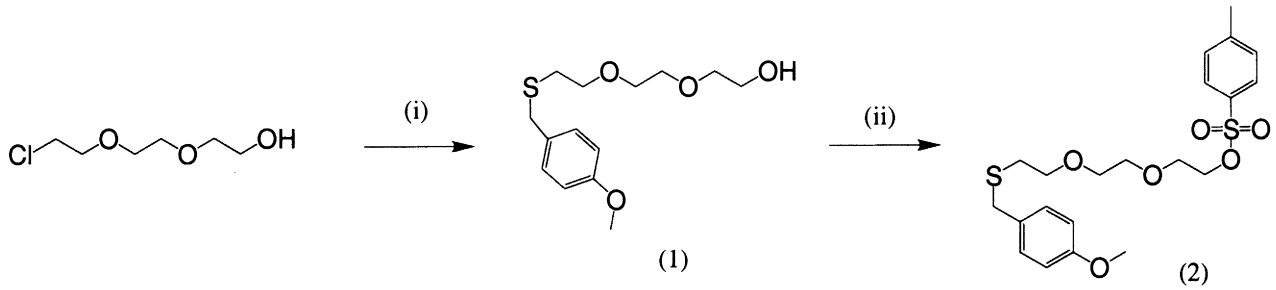
Scheme 1 shows the method developed for the synthesis of the polyethylene glycol derivative. 2-[(2-(2-chloroethoxy)-ethoxy]ethanol undergoes a nucleophilic displacement by *para*-methoxybenzyl thiol resulting in the thioether (**1**) in a 71% yield. The alcohol is converted to a tosylate by stirring in pyridine with an equimolar amount of tosyl chloride[8] giving the tosylate (**2**) in a 72% yield.

Scheme 2 shows the methods used for the synthesis of melatonin and serotonin ligands. tBOC protected serotonin[13] was coupled to the linker arm in the presence of potassium carbonate. The reaction was slow, requiring at least 150 hours to go to completion. A large excess of base was required to obtain a 60% yield of (**5**). When a phthalimido[11] protecting group was used instead of tBOC, the protected serotonin could be coupled with the linker arm using 3 equivalents of caesium carbonate in refluxing acetone over a 24 hour period, resulting in a 60 % yield of (**3**).

The protecting group was removed to give (**4**) using either trifluoroacetic acid[12] or hydrazine to give yields of 92% and 51% of compound (**4**) respectively. The melatonin derivative was synthesised by acylating the intermediate (**4**) with acetyl chloride to give a 70% yield of (**6**). The thiol was deprotected by using mercury (II) acetate and hydrogen sulfide[14] to give (**7**) in a 27% yield. Compound (**4**) was deprotected in a similar manner to give the serotonin derivative (**8**), in a 39% yield. This compound was found to have an EC_{50} = 115μM for the serotonin transporter[9] when it was conjugated to core shells. These compounds may be attached to core shells using the standard ligand exchange reaction described by Dabbousi *et al.*[10]

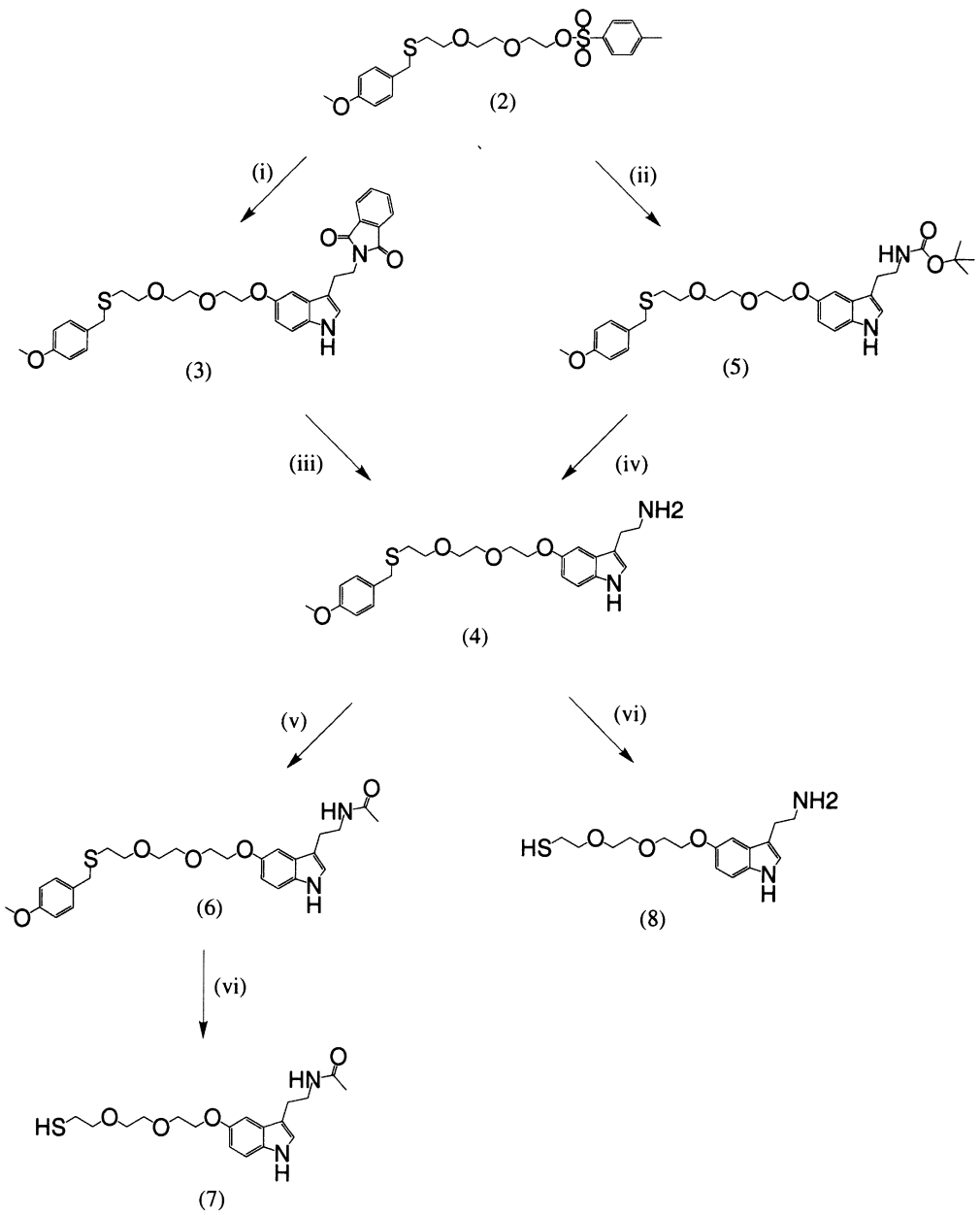
We would like to thank Vanderbilt University for supporting this research in part by providing an intramural discovery grant and we would also like to acknowledge the National Institute of Health for provision of grant # 5R03191-161874-02. N. Sadiqi also acknowledges the support of a Vanderbilt University summer research program fellowship.

Techniques used: microanalysis, LRMS, HRMS, ^{1}H and ^{13}C NMR.



Scheme 1 (i) 2-2-2-chloroethoxy(ethoxy)ethanol, Na, ethanol, *para*-methoxy toluene thiol, reflux 24 hours; (ii) pyridine, *para* toluene sulfonyl choride room temperature 24 hours.

* To receive any correspondence E-mail: sjr@femto.cas.vanderbilt.edu



Scheme 2 (i) N,N-Phthalimido-2-(5-hydroxy-1H-indole-3-yl) ethylamine, CsCO3, acetone, reflux 24 hours; (ii) 3-[2-N-(*tert*-butoxycarbonyl)amino]-ethyl]-1H-indole-5-ol, potassium carbonate, acetone, reflux 240 hours; (iii) hydrazine hydrate, ethanol; (iv) trifluoroacetic acid, room temperature, 2 hours; (v) acetyl chloride, triethylamine, dichloromethane, 18 hours room temperature; and (vi) trifluoro acetic acid, anisole, mercury (II) acetate, hydrogen sulfide.

Received 10 September 2001; accepted 24 February 2002
Paper 01/1050

References

1 Z. Chunyang, M. Hui,. S. Nie, D. Yao, J. Lei and C. Dieyan, *The Analyst*, 2000, **125**, 1029.
2 I. Sondi, O. Silman, S. Koester and E. Matijev, *Langmuir*, 2000, **16**, 3107.
3 M. Bruchez, M. Moronne, P. Gin, S. Weiss and A. P. Alivisatos, *Science*, 1998, **281**, 2013.
4 W.C.W. Chan and S. Nie, *Science*, 1998, **281**, 2016.
5 H. Lang, C. Duschl and H. Vogel, *Langmuir*, 1994, **10**, 1994.
6 N. Boden, R. Bushby, Q. Liu, S. Evans, A. Toby, A. Jenkins, P. Knowles and R. Miles, *Tetrahedron*, 1998, **54**, 11537.
7 T.W. Greene, *Protective groups in organic chemistry,* Ed. J.F.W. McOmie, Plenum Press, New York, 1973, pp. 197-198.
8 C. Almansa, A. Mayo and F. Serratosa, *Tetrahedron* 1991, **47**, 5867.
9 S. J. Rosenthal, I. D. Tomlinson, S. Schroeter, E. M. Adkins, L. Swarford, Y. Wang, L. J. Defelice and R.D. Blakely, *J. the Am. Chem. Soc.,* in press
10 B.O. Dabbousi, V. Rodriguez, F.V. Mikulec, J.R. Heine, H. Mattoussi, R. Ober, K.F. Jensen and M.G. Bawendi, *J. Phys. Chem.* B, 1997, **101**, 9463-9475
11 N. Boden, R. Bushby, S. Clarkson, S. Evans, P. Knowles and S. Marsh, *Tetrahedron*, 1997, **31**, 10939
12 T. Barf, P. Boer, H. Wikstrom, S. Peroutka, K. Svensson, M. Ennis, N. Ghazal, J. McGuire and M. Smith, *J. Med. Chem.,* 1996, **39**, 4717.
13 S. Halazy, M. Perez, C. Fourrier, I. Pallard, P. Petrus, C. Palmer, G. John, J. Valentin, R. Bonnafous and J. Martinez, *J. Med. Chem.*, 1996, **39**, 4920.
14 R. Glennon, S. Hong, M. Bondarev, H. Law, S. Rakhit, P. Power, E. Fan, D. Kinneau, R. Kamboj, M. Teitler, K. Gerrick-Davis and C. Smith, *J. Med. Chem.,* 1996, **39**, 314.
15 E. Gordon, J. Godfrey, N. Delaney, M. Asaad, D. Von Langen and D. Cushman, *J. Med.Chem.*, 1998, **31**, 2199.