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New iodoreboxetine analogues for SPECT imaging of the noradrenaline transporter

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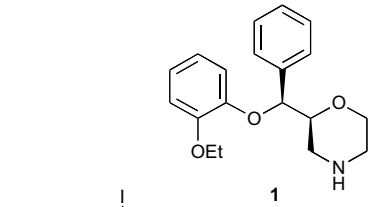
ABSTRACT

A new route for the stereoselective synthesis of iodinated reboxetine analogues has been developed for the generation of SPECT imaging agents for the noradrenaline transporter (NAT). (2*S*,3*S*)- and (2*R*,3*R*)-iodoreboxetine were prepared and biological testing against various mono-amine transporters showed these compounds to be potent and selective for NAT.

The noradrenaline transporter (NAT), which is located at the pre-synaptic terminal of noradrenergic neurons, plays a key role in noradrenergic neurotransmission by regulating the concentration of noradrenaline in the synaptic cleft via a re-uptake mechanism.[1,2] Alterations in synaptic levels of noradrenaline have been implicated in a number of neuropsychiatric and neurodegenerative disorders such as depression, attention-deficit/hyperactivity disorder, anxiety and Alzheimer's disease. In addition, NAT is a major target for drugs targeted at a range of psychiatric conditions.[3–6]

Non-invasive imaging techniques such as single photon emission computed tomography (SPECT) or positron emission tomography (PET) could provide useful tools for determining the status of NAT in patients with neuropsychiatric or neurodegenerative disorders and the assessment of drug occupancy of the transporter in vivo. Most of the studies involved in achieving such a goal have focused on the development of radioiodinated analogues of reboxetine **1**, the well-known noradrenaline re-uptake inhibitor,[7] for in vivo SPECT imaging.[8–10] As most of the general NAT studies of reboxetine analogues have focused on the (2*S*,3*S*)-**1** and the (2*R*,3*R*)-stereoisomers,[8,9,11] we became interested in investigating iodoanalogues of the (2*S*,3*R*)- and the (2*R*,3*S*)-stereoisomers as possible SPECT imaging agents for NAT. This led to the development of a stereoselective synthesis of (2*S*,3*R*)-iodoreboxetine **2** and (2*R*,3*S*)-iodoreboxetine **3** and in vitro testing of these compounds with homogenised rat brain identified **3** as the most potent for NAT with a K_i value of 58.2 nM.[10]



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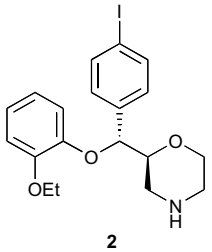

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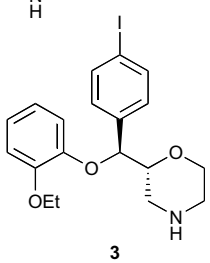

3

In an effort to gain further insight into how the stereochemistry of reboxetine affects binding affinity with NAT and to produce a more potent iodoanalogue for SPECT imaging, the preparation of the two other stereoisomers of **2** and **3**, (2*S*,3*S*)-iodoreboxetine **4** and (2*R*,3*R*)-iodoreboxetine **5** was proposed. In this Letter, we now report the stereoselective synthesis of iodoanalogues **4** and **5** and the screening of these compounds for affinity with NAT, the serotonin transporter (SERT) and the dopamine transporter (DAT).

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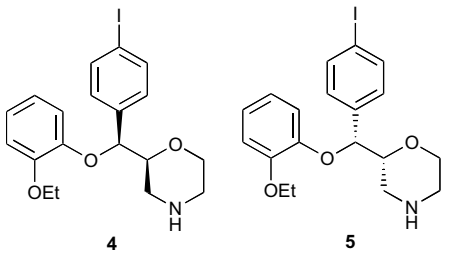
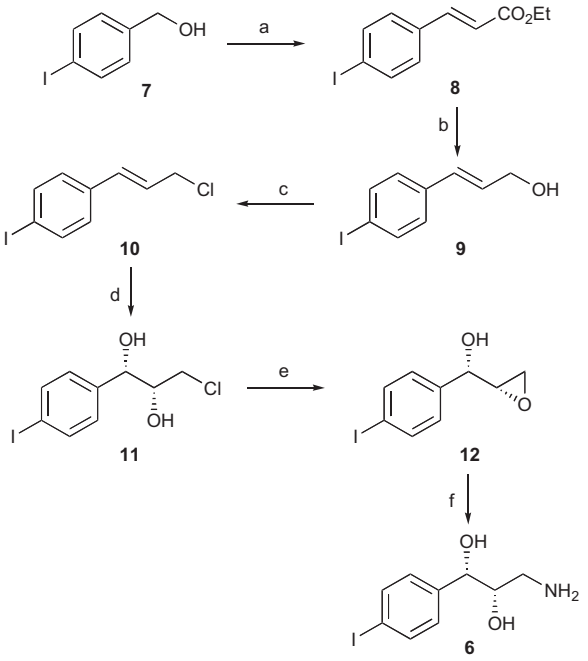


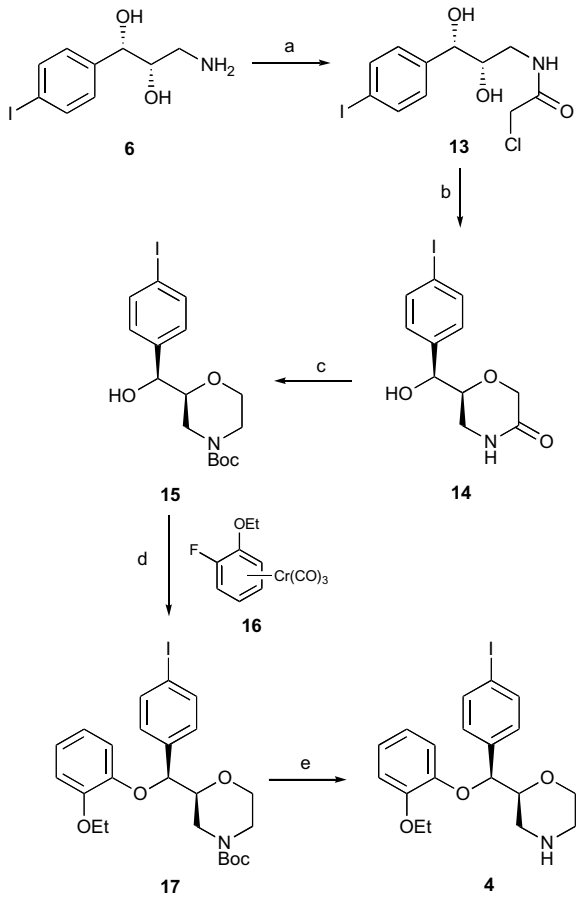
The first stage of this study required the development of a stereoselective synthesis of (2*S*,3*S*)-iodoreboxetine **4**. The first key intermediate in this route, amino alcohol **6** was prepared in six steps as shown in Scheme 1. 4-Iodobenzyl alcohol **7** was used as the starting material and converted in a one-pot Swern oxidation/Horner–Wadsworth–Emmons reaction under Masumune–Roush conditions to *E*-α,β-unsaturated ester **8** in 99% yield.[12] Reduction of **8** using DIBAL-H gave allylic alcohol **9** in 99% yield. We planned to create the stereogenic centres of **6** using a Sharpless asymmetric dihydroxylation.[13] Sharpless and co-workers have shown that allylic chlorides are excellent substrates for the asymmetric dihydroxylation reaction.[14] Thus, allylic alcohol **9** was converted to the corresponding allylic chloride **10** using triphenylphosphine and *N*-chlorosuccinimide and this was subjected to an asymmetric dihydroxylation using AD-mix-α which gave diol **11** in 84% yield and in an excellent 98% e.e.[15]

Treatment of **11** with sodium hydroxide led to efficient formation of epoxide **12** and this underwent a regioselective ring-opening reaction with aqueous ammonia solution to give the desired amino alcohol **6** in 90% yield.

The next stage of the synthesis of (2*S*,3*S*)-iodoreboxetine **4** required the formation of the morpholine ring and a similar approach as previously described for our synthesis of **2** and **3** was utilised (Scheme 2).[10] Thus, reaction of amino alcohol **6** with chloroacetyl chloride gave amide **13** and ring closure was then effected



Scheme 1. Reagents and conditions: (a) i—$(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C; ii—triethyl phosphonoacetate, LiCl, DBU, MeCN, 99% over two steps; (b) DIBAL-H (2.2 equiv), Et_2O, −78 °C to RT, 99%; (c) *N*-chlorosuccinimide, PPh_3, CH_2Cl_2, 0 °C, 69%; (d) AD-Mix-α, $MeSO_2NH_2$, $NaHCO_3$, *t*-BuOH, H_2O, 0 °C, 84%; (e) NaOH, THF, 0 °C, 98%; (f) 25% NH_3 (aq), MeCN, 90%.



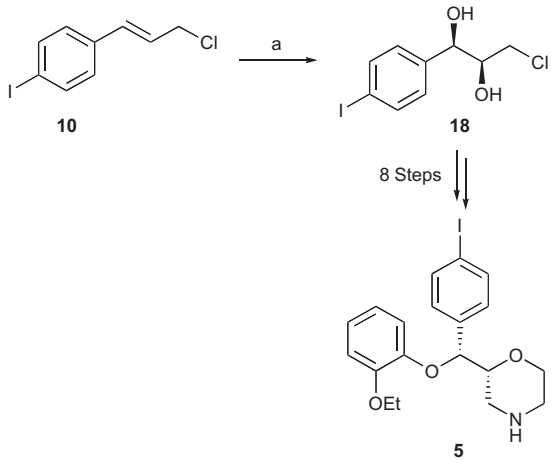
Scheme 2. Reagents and conditions: (a) chloroacetyl chloride, NEt_3, MeCN, −10 °C to RT, 57%; (b) sodium *tert*-butoxide, *t*-BuOH, 40 °C, 65%; (c) i—$BH_3.Me_2S$, THF, 0 °C to RT; ii—Boc_2O, Et_3N, DMAP (cat.), CH_2Cl_2, 40% over two steps; (d) NaH, DMF then I_2, 63%; (e) TFA, CH_2Cl_2, 60%.

using sodium *tert*-butoxide which gave morpholinone **14** in 51% yield over the two steps.[16] Reduction of **14** was then carried out using borane-dimethylsulfide and the resulting amine was Boc-protected to give **15** in 40% yield over the two steps.

Our initial strategy for introducing the 2-ethoxyphenyl group of **4** involved bromination of the benzyl alcohol component of **15**, followed by nucleophilic displacement with 2-ethoxyphenol. While bromination of **15** using carbon tetrabromide and polymer supported triphenylphosphine proceeded smoothly, all attempts at displacement of the bromide using 2-ethoxyphenol gave only complex mixtures of products.[11b] Instead, a nucleophilic aromatic substitution reaction previously described by Tamagnan and co-workers involving chromium tricarbonyl activated 2-ethoxyfluorophenol **16** was utilised and this gave **17** in 63% yield.[17] Finally, removal of the Boc-protecting group under standard conditions gave (2*S*,3*S*)-iodoreboxetine **4** in 60% yield.

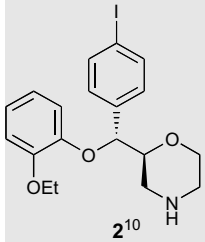
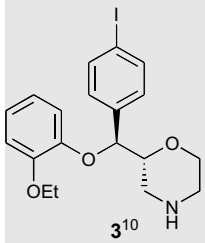
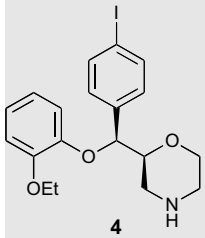
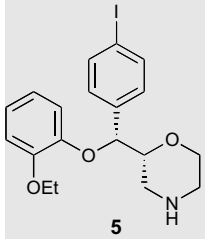
This approach was also used for the synthesis of (2*R*,3*R*)-iodoreboxetine **5** (Scheme 3). In this case, allylic chloride **10** was subjected to an asymmetric dihydroxylation using AD-mix-β, which gave the corresponding diol **18** in 62% yield and in an excellent 98% e.e. Diol **18** was then converted to (2*R*,3*R*)-iodoreboxetine **5** in 8-steps as described for compound **4**.

The binding of compounds **4** and **5** at noradrenaline, serotonin and dopamine transporters was evaluated in vitro using homogenates of rat brain (Table 1).[18] (2*S*,3*S*)-Analogues of reboxetine generally have high affinity for NAT and can be up to 10- to 100-fold more selective than the corresponding (2*R*,3*R*)-stereoisomer.[8,11d]



Scheme 3. Reagents and conditions: (a) AD-Mix-β, $MeSO_2NH_2$, $NaHCO_3$, *t*-BuOH, H_2O, 0 °C, 62%.

Table 1
Binding affinity of reboxetine analogues **4** and **5** with NAT, SERT and DAT (NAT data for **2** and **3** included for comparison)[10]

Compound	NAT K_i (nM)[a]	SERT K_i (nM)[b]	DAT K_i (nM)[b]
 **2**[10]	320.8 ± 9.0	–	–
 **3**[10]	58.2 ± 9.4	–	–
 **4**	53.8 ± 2.7	2793 ± 480	1457 ± 150
 **5**	64.0 ± 2.4	646 ± 142	716 ± 20

[a] K_i values are the mean of 3 separate determinations.
[b] K_i values are the mean of 2 separate determinations.

As expected, (2*S*,3*S*)-iodoreboxetine **4** has high affinity for NAT with a K_i value of 53.8 nM. Surprisingly, (2*R*,3*R*)-iodoreboxetine **5** has also significant potency for NAT with a similar K_i value of 64.0 nM. Our previous work with stereoisomers, **2** and **3** and our result for (2*S*,3*S*)-analogue **4** shows that the 3*S*-stereogenic centre is required for high affinity with NAT.[10] The fact that (2*R*,3*R*)-stereoisomer also binds to NAT with high affinity, strongly suggests that this compound must bind in a different manner to the other stereoisomers. The addition of a large iodine atom to the phenyl ring of **5** compared to the parent (2*R*,3*R*)-reboxetine compound, means that the two aromatic rings are now more similar in size and thus, this may allow these moieties to alternate binding pockets, producing a similar 3-D shape at the C3-position as with stereoisomers **3** and **4**, resulting in similar levels of potency.

Compounds **4** and **5** are significantly more selective for NAT than the other mono-amine transporters. In particular, (2*S*,3*S*)-iodoreboxetine **4** is approximately 50-fold and 25-fold more selective for NAT than SERT and DAT respectively and thus, future analogues of **4** may have both the potency and selectivity required for developing an effective imaging agent for NAT.

In conclusion, we have developed a new stereoselective route for the synthesis of (2*S*,3*S*)-iodoreboxetine **4** and (2*R*,3*R*)-iodoreboxetine **5** and have shown these compounds to have high affinity and selectivity for NAT. The preparation and testing of all four stereoisomers of this iodoreboxetine analogue has generated valuable insight into how the different stereoisomers of reboxetine bind to NAT and our work has revealed that the (2*R*,3*S*)- and (2*S*,3*S*)-stereoisomers in particular, have the highest affinity for NAT. Work is currently underway to further confirm this hypothesis and to generate more potent and selective analogues around a (2*R*,3*S*)- and (2*S*,3*S*)-morpholine backbone for the development of an effective SPECT imaging agent for NAT.

Acknowledgements

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Supplementary data

Experimental procedures and spectroscopic data for all compounds synthesised as well as details for competition binding assays. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.041.

References and notes

1. (a) Amara, S. G.; Kuhar, M. J. *Annu. Rev. Neurosci.* **1993**, *16*, 73; (b) Blakely, R. D.; Bauman, A. L. *Curr. Opin. Neurobiol.* **2000**, *10*, 328.
2. Zhu, M.-Y.; Ordway, G. A. *J. Neurochem.* **1997**, *68*, 134.
3. Spillmann, M. K.; Van der Does, A. J. W.; Rankin, M. A.; Vuolo, R. D.; Alpert, J. E.; Nierenberg, A. A.; Rosenbaum, J. F.; Hayden, D.; Schoenfeld, D.; Fava, D. *Psychopharmacology* **2001**, *155*, 123.
4. Biederman, J.; Spencer, T. *Biol. Psychiatry* **1999**, *46*, 1234.
5. Hisamichi, S.; Moon, A. Y.; Aoyagi, A.; Hara, T.; Abe, K.; Yamazaki, R.; Kumagae, Y.; Naruto, S.; Koyama, K.; Marumoto, S.; Tago, K.; Toda, N.; Takami, K.; Yamada, N.; Ori, M.; Kogen, H.; Kaneko, T. *J. Pharmacol. Sci.* **2003**, *93*, 95.
6. Tejani-Butt, S. M.; Yang, J. X.; Zaffar, H. *Brain Res.* **1993**, *631*, 147.
7. Hajos, M.; Fleishaker, J. C.; Filipiak-Reisner, J. K.; Brown, M. T.; Wong, E. H. F. *CNS Drug Rev.* **2004**, *10*, 23.
8. Kanegawa, N.; Kiyono, Y.; Kimura, H.; Sugita, T.; Kajiyama, S.; Kawashima, H.; Ueda, M.; Kuge, Y.; Saji, H. *Eur. J. Nucl. Med. Mol. Imag.* **2006**, *33*, 639.
9. Tamagnan, G. D.; Brenner, E.; Alagille, D.; Staley, J. K.; Haile, C.; Koren, A.; Early, M.; Baldwin, R. M.; Tarazi, F. I.; Baldessarini, R. J.; Jarkas, N.; Goodman, M. M.; Seibyl, J. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 533.
10. Jobson, N. K.; Spike, R.; Crawford, A. R.; Dewar, D.; Pimlott, S. L.; Sutherland, A. *Org. Biomol. Chem.* **2008**, *6*, 2369.
11. For recent examples, see: (a) Wilson, A. A.; Johnson, D. P.; Mozley, D.; Hussey, D.; Ginovart, N.; Nobrega, J.; Garcia, A.; Meyer, J.; Houle, S. *Nucl. Med. Biol.* **2003**, *30*, 85; (b) Boot, J.; Cases, M.; Clark, B. P.; Findlay, J.; Gallagher, P. T.; Hayhurst,

L.; Man, T.; Montalbetti, C.; Rathmell, R. E.; Rudyk, H.; Walter, M. W.; Whatton, M.; Wood, V. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 699; (c) Harding, W. W.; Hodge, M.; Wang, Z.; Woolverton, W. L.; Parrish, D.; Deschamps, J. R.; Prisinazano, T. E. *Tetrahedron: Asymmetry* **2005**, *16*, 2249; (d) Fish, P. V.; Deur, C.; Gan, X.; Greene, K.; Hoople, D.; Mackenny, M.; Para, K. S.; Reeves, K.; Ryckmans, T.; Stiff, C.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett* **2008**, *18*, 2562.

12. (a) Ireland, R. E.; Norbeck, D. W. *J. Org. Chem.* **1985**, *50*, 2198; (b) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masumune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183.
13. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
14. VanHessche, K. P. M.; Wang, Z.-M.; Sharpless, K. B. *Tetrahedron Lett.* **1994**, *35*, 3469.
15. The enantiomeric excess of compounds **11** and **18** was determined by chiral HPLC. See supplementary data for full details.
16. Siddiqui, S. A.; Narkhede, U. C.; Lahoti, R. J.; Srinivasan, K. V. *Synlett* **2006**, 1771.
17. Brenner, E.; Baldwin, R. M.; Tamagnan, G. D. *Org. Lett.* **2005**, *7*, 937.
18. A [^{3}H]nisoxetine displacement assay was used for NAT, a [^{3}H]citalopram displacement assay for SERT and a [^{3}H]WIN-35,428 displacement assay for DAT. See supplementary data for full details.