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# Design and synthesis of (2*R*,3*S*)-iodoreboxetine analogues for SPECT imaging of the noradrenaline transporter

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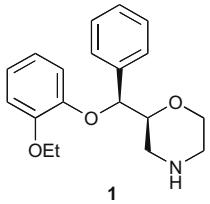
ABSTRACT

A stereoselective 10-step synthesis of iodophenoxy analogues of (2*R*,3*S*)-reboxetine has been developed with the aim of generating a new SPECT imaging agent for the noradrenaline transporter (NAT). In vitro testing of these compounds against various mono-amine transporters showed an *ortho*-iodophenoxy analogue to have excellent affinity ($K_i$ 8.4 nM) and good selectivity for NAT.



The noradrenaline transporter (NAT) is a membrane spanning protein situated on the pre-synaptic terminal of noradrenergic neurons. Its main function is to regulate the concentration of noradrenaline in the synaptic cleft via a re-uptake mechanism.[1,2] Recent research has shown that a reduction in the synaptic levels of noradrenaline is associated with various neuropsychiatric and neurodegenerative disorders such as attention-deficit/hyperactivity disorder, anxiety, Alzheimer's disease and depression and thus, NAT is a major target for the development of drugs for these conditions.[3–6]

Non-invasive imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been proposed as important tools for determining the link between NAT and these neuropsychiatric and neurodegenerative disorders. These techniques could also be used for the assessment of drug occupancy of the transporter in vivo. To utilise PET and SPECT in this way requires the development of highly selective radiolabelled molecular probes. Recent work has focused on the development of probes based on (2*S*,3*S*)-reboxetine **1**, the well-characterised NAT inhibitor.[7] Development of small libraries of iodinated analogues of reboxetine, by the Saji and Tamagnan groups identified analogue **2** ($K_i$ 0.84–4.22 nM) as having significant potential as an imaging agent of NAT.[8,9] With the aim of understanding the stereochemical pharmacology of NAT for the development of novel SPECT tracers, we recently reported the synthesis and in vitro testing of four stereoisomers of an iodophenylreboxetine analogue.[10,11] The results of the biological testing showed that while the (2*S*,3*S*)-iodophenylreboxetine analogue **3** had as expected, significant affinity for NAT ($K_i$ 53.8 nM), the (2*R*,3*S*)-analogue **4** was of similar potency ($K_i$ 58.2 nM). The outcome of this work revealed that (2*R*,3*S*)-analogues of reboxetine should also be considered when designing high affinity molecular probes for NAT.

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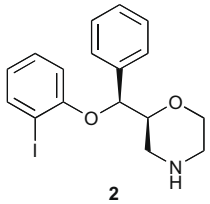


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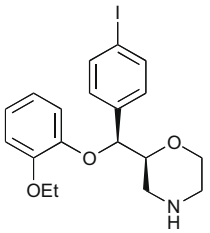


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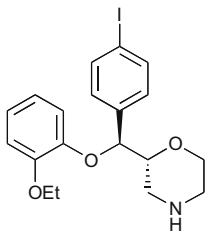


**4**

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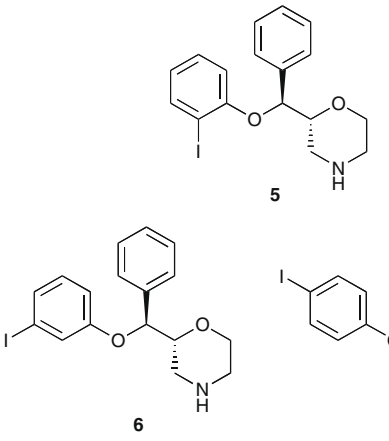
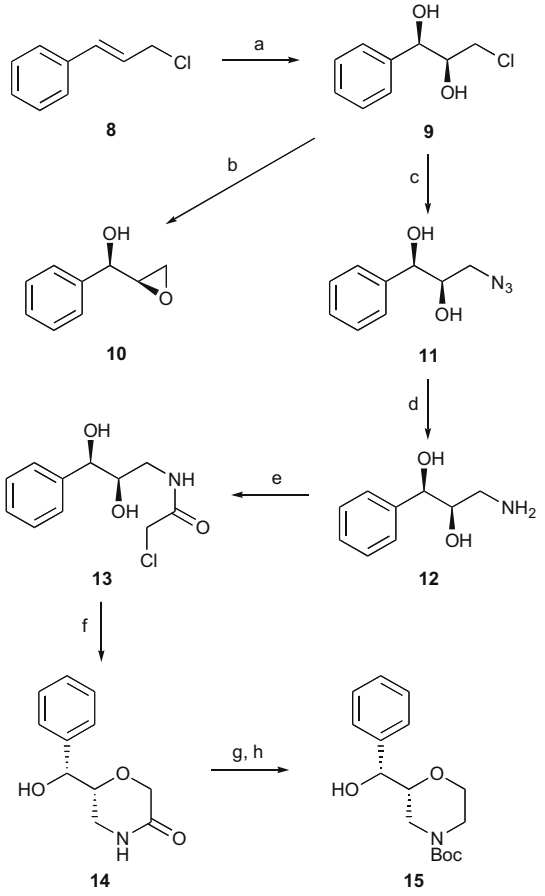
In an effort to identify new high affinity SPECT tracers for the imaging of NAT, we were interested in preparing the (2*R*,3*S*)-stereoisomer **5** of the iodophenoxyreboxetine analogue **2** reported by the Saji and Tamagnan groups.[8,9] Our aim was to develop a flexible synthetic route to **5** that would also permit the preparation of *meta*- and *para*-iodophenoxy isomers **6** and **7**, thus allowing us to probe the structure–activity-relationship of the iodophenoxy moiety. Herein, we now report a 10-step stereoselective synthesis of **5**, **6** and **7**. In vitro testing of all three compounds for affinity with NAT, the serotonin transporter (SERT) and the dopamine transporter (DAT) is also described.




In our previous synthesis of a (2*R*,3*S*)-reboxetine analogue, a Sharpless asymmetric epoxidation[12] was used to establish the key stereogenic centres followed by the preparation of an amino alcohol which was eventually used to form the morpholine ring.[10] While this approach did produce the target compound, introduction of diversity occurred early in the route, effectively restricting the number of analogues which could be produced. In this current study, we have developed an alternative, more flexible approach to (2*R*,3*S*)-reboxetine analogues using a Sharpless asymmetric dihydroxylation[13] to create the key stereogenic centres followed by subsequent formation of the morpholine ring. The final stage uses an activated mesylate intermediate for the concurrent introduction of the iodophenoxy moieties and inversion of the stereochemical configuration at the C-3 position to give the desired (2*R*,3*S*)-analogues. Thus, cinnamyl chloride **8** was subjected to a Sharpless asymmetric dihydroxylation using AD-mix-β to give **9** in 66% yield and in an excellent 99% ee (Scheme 1).[14] Diol **9** was then treated with sodium hydroxide, forming the epoxide **10** in 98% yield. During our previous synthesis of (2*S*,3*S*)- and (2*R*,3*R*)-iodophenylreboxetine analogues, it was shown that 2,3-epoxy-1-(4-iodophenyl)propan-1-ols could react with ammonia in a highly regioselective manner, resulting in attack at the least hindered end of the epoxide to give the corresponding amino alcohol.[11] However, a similar reaction with epoxide **10** gave both amino alcohol regioisomers as observed by $^1$H NMR spectroscopy and while this mixture could undergo a transformation with chloroacetyl chloride to give desired intermediate **13**, the isolated yield of **13** was only 25% over the two steps. Srinivasan and co-workers in their synthesis of (2*S*,3*S*)-reboxetine, prepared an analogous amino alcohol via an azide intermediate.[15] In a similar manner, **9** was reacted with sodium azide to give **11** in 85% yield. Catalytic hydrogenation of azide **11** with 10% palladium on carbon at atmospheric pressure gave the key amino alcohol **12** in 90% yield. Amine **12** was then acetylated with chloroacetyl chloride and the morpholinone ring was formed by subsequent reaction with potassium *tert*-butoxide. The

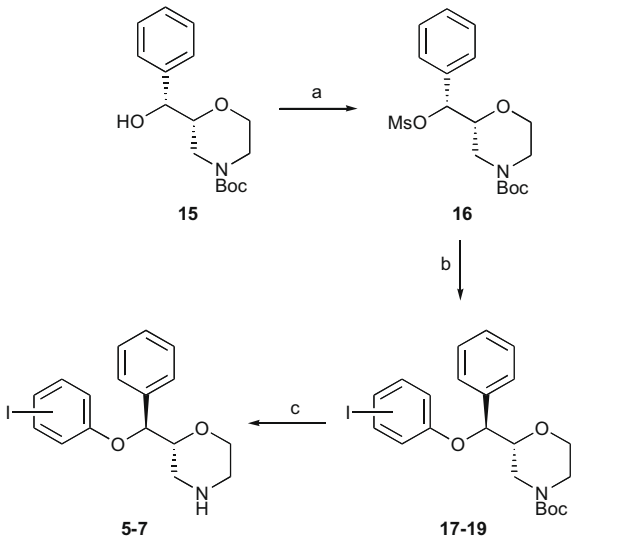



**Scheme 1.** Reagents and conditions: (a) AD-Mix-β, $MeSO_2NH_2$, $NaHCO_3$, *t*-BuOH, $H_2O$, 0 °C, 66%; (b) NaOH, THF, 0 °C, 98%; (c) $NaN_3$, DMF, Δ, 85%; (d) 10% Pd/C, $H_2$, EtOAc, 90%; (e) chloroacetyl chloride, $Et_3N$, MeCN, −10 °C to rt, 52%; (f) sodium *tert*-butoxide, *t*-BuOH, 40 °C, 58%; (g) $BH_3.Me_2S$, THF, 0 °C to rt, 78%; (h) $Boc_2O$, $Et_3N$, DMAP (cat.), $CH_2Cl_2$, 56%.

morpholinone ring was reduced using the borane–dimethylsulfide complex and the resulting amine was Boc-protected to give **15** in good yield over the two steps.

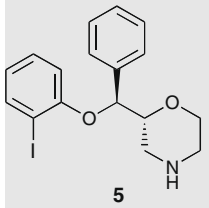
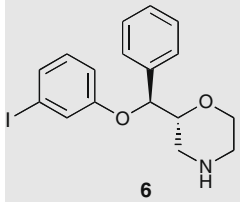
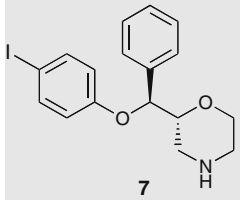
The last stage of the synthesis of **5**, **6** and **7** required the introduction of the iodophenol moieties with inversion of configuration at the C-3 position. This was achieved by activating alcohol **15** as a mesylate under standard conditions (Scheme 2). Mesylate **16** was then reacted with 2-iodo, 3-iodo and 4-iodophenol in the presence of caesium carbonate to give the desired (2*R*,3*S*)-coupled products **17**–**19** with complete inversion of configuration at the C-3 position.[16] Removal of the Boc-protecting groups of **17**–**19** with TFA then gave the desired targets **5**–**7** in good overall yields.

In vitro competition binding of compounds **5**, **6** and **7** at NAT, SERT and DAT was carried out using homogenates of rat brain (Table 1).[17] The *ortho*-iodophenoxy analogue **5** showed excellent affinity for NAT with a $K_i$ value of 8.4 nM and with a 6-fold and 63-fold selectivity compared to SERT and DAT, respectively. This result again shows the suitability of (2*R*,3*S*)-analogues of reboxetine for the development of high affinity agents of NAT. The *meta*- and *para*-iodophenoxy analogues, **6** and **7**, showed considerably less affinity for NAT compared to **5**. The high affinity of **5** relative to **6** and **7** is predictable as reboxetine **1** and other analogues that show high affinity for NAT all have *ortho*-phenoxy substitutents.[7–9,18,19] The significantly high difference in affinity between **5** and **6** (202-fold difference) clearly shows that a binding pocket is available to bind specifically *ortho*-substituents and even a slight shift to the *meta*-position results in a dramatic loss of affinity. While




**Scheme 2.** Reagents and conditions: (a) $MeSO_2Cl$, $Et_3N$, DMAP (cat.), $CH_2Cl_2$, 88%; (b) 2-iodophenol, 3-iodophenol or 4-iodophenol, $Cs_2CO_3$, Δ, 1,4-dioxane, (**17**, 61%; **18**, 32%; **19**, 52%); (c) TFA, $CH_2Cl_2$ (**5**, 76%; **6**, 80%; **7**, 55%).

**Table 1**
Binding affinity of (2*R*,3*S*)-reboxetine analogues **5**, **6** and **7** with NAT, SERT and DAT

| Compound | NAT $K_i$ (nm) | SERT $K_i$ (nm) | DAT $K_i$ (nm) |
|---|---|---|---|
|  **5** | 8.4 ± 1.7[a] | 51.5 ± 8.4 | 525.9 ± 125.5 |
|  **6** | 1700 ± 500 | 154.0 ± 12.0 | 1900 ± 600 |
|  **7** | 1100 ± 200 | 34.5 ± 1.7 | 2100 ± 300 |

[a] Calculated from five independent experiments. All other assays calculated from three independent experiments.

*para*-iodophenoxy analogue **7** showed low affinity for NAT, it did show high affinity ($K_i$ 34.5 nM) and high selectivity for SERT and thus, further analogues of **7** may find application in the SPECT imaging of this receptor.

In summary, a stereoselective 10-step synthesis of (2*R*,3*S*)-iodophenoxyreboxetine analogues has been achieved using a Sharpless asymmetric dihydroxylation to establish the key stereogenic centres. The final stage of this route involved the flexible and stereospecific introduction of various iodophenoxy moieties. While this approach has similar overall yields compared to our previous stereoselective syntheses of iodinated reboxetine analogues, its main advantage is that it permits the introduction of diversity at a much later stage of the synthetic route (e.g., penultimate step) and thus, allows easier preparation of small libraries of compounds. More importantly, in vitro testing of these compounds has identified *ortho*-iodophenoxy analogue **5** as having excellent affinity for NAT and therefore, significant potential for development as a SPECT tracer for this receptor. The preparation of *meta*- and *para*-iodophenoxy analogues **6** and **7** has allowed us to probe the structure–activity-relationship of the iodophenoxy moiety of these compounds and the results have emphasised the requirement of *ortho*-substituted analogues for high affinity with this receptor. The radiosynthesis and in vivo evaluation of **5** as a SPECT imaging agent for NAT and the development of further analogues of **7** for imaging SERT is currently underway.

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## Supplementary data

Supplementary data (Experimental procedures and spectroscopic data for all compounds synthesised as well as details for competition binding assays.) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.064.

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