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SYNTHESIS AND SAR OF DIIODOTYROSINE-DERIVED GLYCINE-SITE *N*-METHYL-*D*-ASPARTATE RECEPTOR LIGANDS

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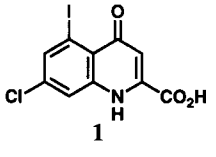
Abstract. A series of analogues of the novel diiodotyrosine derived NMDA glycine-site ligand **(*R*)-4** was prepared in which the aryl substitution, chain length and amino acid groups were varied. The key structural features for binding are the α-amino acid function, having the (*R*) absolute stereochemistry, the 3,5-diiodo substituted aromatic ring and a lipophilic group attached at the phenolic oxygen of the tyrosine moiety.

Antagonists which act at the glycine modulatory site of the *N*-methyl-*D*-aspartate (NMDA) subtype of excitatory amino acid receptor have potential for the treatment of various neurological disorders such as cerebral ischaemia, head injury, epilepsy and schizophrenia.[1,2]

We have developed several classes of glycine antagonists over recent years, exemplified by 5-iodo-7-chlorokynurenic acid (**1**),[3] L-689,560 (**2**),[4] and L-701,324 (**3**).[5] In addition to the design approach which led to the discovery of these milestone compounds, we have also undertaken screening of the Merck sample library for novel lead structures. This resulted in the identification of the racemic 3,5-diiodotyrosine derivative **4** (IC_{50} 0.9μM) as a glycine-site NMDA receptor ligand. Synthesis of both enantiomers of **4** established that affinity for the glycine-site resided exclusively with the (*R*)-enantiomer [**(*R*)-4** IC_{50} 0.43μM, **(*S*)-4** IC_{50} >100μM]. In this communication we disclose the results of structure-activity studies on the novel diiodotyrosine-derived glycine ligand **(*R*)-4**.



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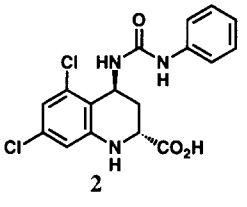

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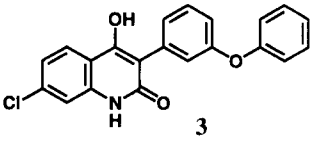

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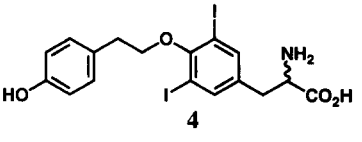
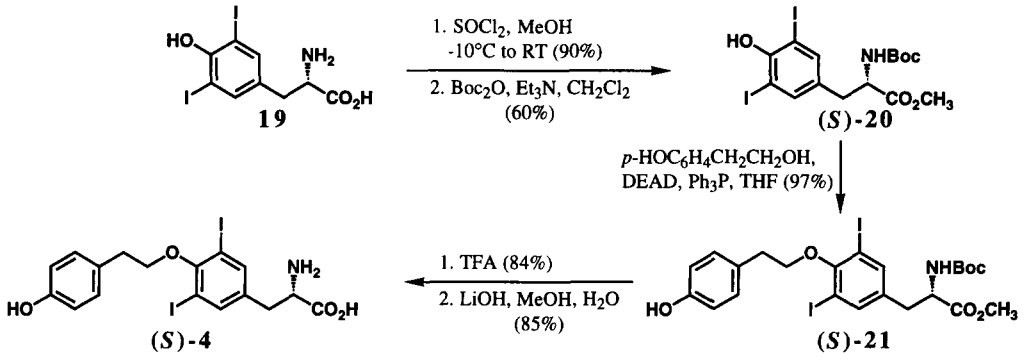


4

Chemistry

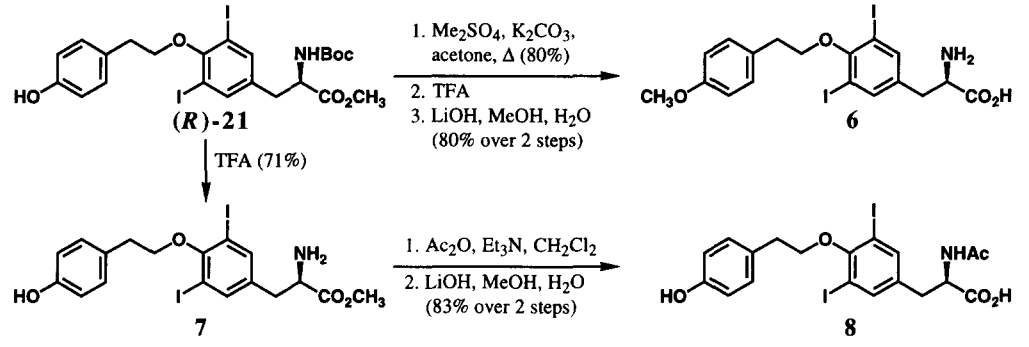
Synthesis of the (*S*)-enantiomer of the lead structure, **(*S*)-4**, was accomplished by the method outlined in Scheme 1. The amino acid moiety of 3,5-diiodo-*L*-tyrosine **19** was masked and the phenolic oxygen alkylated under Mitsunobu conditions[6] to furnish the protected derivative **(*S*)-21** in excellent yield. Deprotection gave **(*S*)-4**. The corresponding (*R*)-isomer, **(*R*)-4**, was synthesised using similar chemistry.

Scheme 1

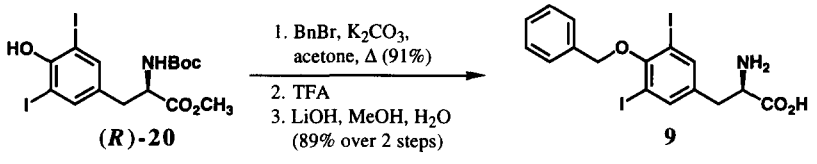


The protected amino acid (*R*)-**21** proved a valuable intermediate for the preparation of three analogues of (*R*)-**4**, the methyl ether **6**, amino ester **7** and amide **8** (Scheme 2). The benzyl compound **9** was prepared by benzyl bromide alkylation of (*R*)-**20** followed by the usual deprotection conditions (Scheme 3).

Scheme 2



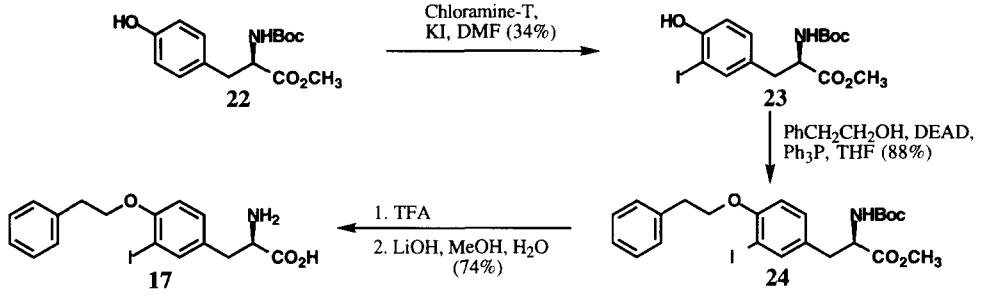
Scheme 3



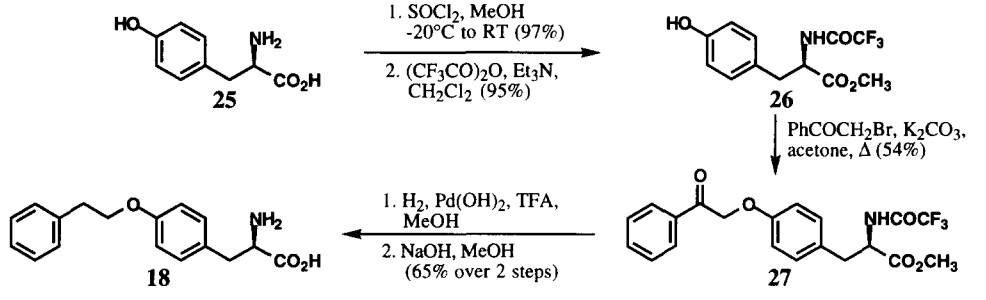
Monoiodination of Boc-*D*-tyrosine methyl ester **22** was carried out by treatment with *N*-chloro-*p*-toluenesulphonamide sodium salt (chloramine T), in the presence of potassium iodide,[7] to afford the iodophenol **23** (Scheme 4). Mitsunobu reaction with phenethyl alcohol proceeded efficiently to give **24**, which was deprotected to afford the required monoiodide **17**. The des-iodo compound **18** was prepared from *D*-tyrosine **25** as shown in Scheme 5.

The thyroid hormone analogues **10**, **11** and **12** were commercially available (Sigma/Aldrich) and the syntheses of compounds **5**, **13**, **14** and **15** were carried out by analogous methods to those described.

Scheme 4



Scheme 5



Results and Discussion

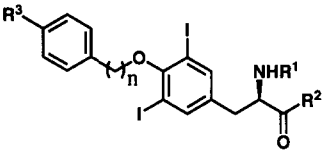
The primary screening assay used for testing compounds was the binding affinity for the NMDA receptor glycine-site *in vitro* as measured by displacement of [^{3}H]L-689,560 binding to rat brain membranes (IC_{50} values).[8]

The glycine-site binding of the diiodotyrosine-derived lead structure **4** was enantioselective for the (*R*)-isomer, **(*R*)-4**, by at least 200-fold over the (*S*)-enantiomer, **(*S*)-4** (IC_{50}'s 0.43 and >100μM respectively). Such enantiospecific binding is well established for glycine ligands, for example the agonists (*R*)-alanine and (*R*)-serine,[9] the partial agonist *R*-(+)-HA-966[10] and the glycine antagonist L-689,560 **2**.[4]

The requirement of the phenol, amine and acid functional groups of **(*R*)-4** for glycine binding was examined by masking each in turn. The phenolic group did not appear to be necessary since the des-hydroxy compound **5** and the methylated analogue **6** had similar binding affinities to **(*R*)-4** (Table 1). However, acetylation of the amine or esterifying the acid group abolished binding affinity, suggesting that the amino acid moiety of the lead structure was an essential requirement for glycine-site binding. Next, the chain length between the aromatic groups was investigated by truncating one methylene at a time. The lower homologue of **5**, *i.e.* **9**, retained similar binding, indicating significant tolerance in the relative positioning of the two aromatic rings. Truncating by two carbon atoms, *i.e.* to give 3,5-diiodo-*D*-thyronine **10**, reduced affinity by an order of magnitude.

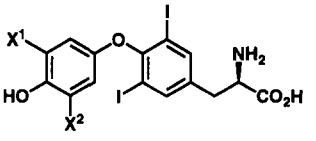
Given the glycine binding observed for 3,5-diiodo-*D*-thyronine **10**, two further thyroid hormone analogues were tested (Table 2). Substitution of the second phenyl ring with first one iodine atom (3,3',5-triiodo-*D*-thyronine **11**) then two (*D*-thyroxine **12**) produced a stepwise increase in binding affinity.

Table 1 - Investigation of functional groups and chain length



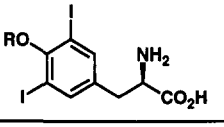
Compound	R^1	R^2	R^3	n	[^{3}H]L-689,560 IC_{50} (μM)
(*R*)-**4**	H	OH	OH	2	0.43
5	H	OH	H	2	0.51
6	H	OH	OCH_3	2	0.54
7	H	OCH_3	OH	2	~100
8	CH_3CO	OH	OH	2	>100
9	H	OH	H	1	0.79
10	H	OH	OH	0	4.1

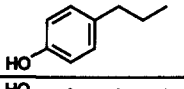
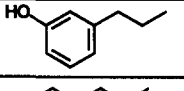
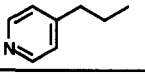
Table 2 - Thyroid hormone analogues



Compound	X^1	X^2	[^{3}H]L-689,560 IC_{50} (μM)
10	H	H	4.1
11	I	H	1.5
12	I	I	0.33

Table 3 - Replacement of the *p*-hydroxyphenethyl moiety

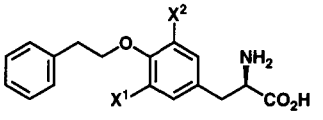


Compound	R	[^{3}H]L-689,560 IC_{50} (μM)
(*R*)-**4**		0.43
13		0.35
14		0.46
15	Et	1.5
16	H	~100

A number of further modifications to the *p*-hydroxyphenethyl portion of the lead (***R***)-**4** were investigated (Table 3). Thus, the *m*-hydroxyphenethyl **13** and 4-pyridylethyl **14** compounds were found to have equivalent binding affinity to the *p*-hydroxyphenethyl lead (***R***)-**4**. Deletion of the *p*-hydroxyphenyl ring to give the ethyl compound **15** had a surprisingly modest effect on the binding affinity, indicating a relatively small contribution to glycine binding of this portion of (***R***)-**4**. Thus, the >200-fold reduction of affinity observed for 3,5-diiodo-*D*-tyrosine **16** was striking by comparison, although this may be due to an unfavourable polar interaction of the phenol group at the glycine binding site.

The importance of the iodine atoms to binding was investigated by synthesis of the mono- and des-iodo compounds, **17** and **18** respectively (Table 4). Each removal of an iodine resulted in an approximately ten-fold reduction in affinity, indicating a key role for the 3,5-diiodo substitution pattern in the lead structure.

Table 4 - Function of the iodine atoms

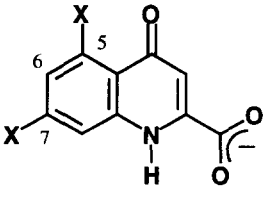


Compound	X^1	X^2	[^{3}H]L-689,560 IC_{50} (μM)
5	I	I	0.51
17	I	H	7.3
18	H	H	~100

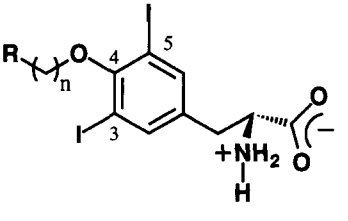
A number of the high affinity glycine-site ligands identified in this study were shown to be antagonists in a functional assay. For example, *in vitro* antagonist potencies (K_b values) of (***R***)-**4** (K_b 2.2μM), **13** (K_b 2.5μM) and **14** (K_b 0.67μM) were determined by blockade of NMDA-induced depolarisations on rat cortical slices.[11]

It is intriguing to consider how the glycine-site binding of this novel series of diiodotyrosine-derived ligands can be rationalised with proposed pharmacophore models for other classes of glycine antagonists, such as the kynurenic acids, *e.g.* **1**,[3] and the tetrahydroquinolines, exemplified by L-689,560 (**2**).[2,4] Given that the kynurenic acids display highest affinity with 5,7-dihalo substitution,[3] it is perhaps compelling to consider the obvious overlay with the diiodotyrosine series (Figure 1).

Figure 1

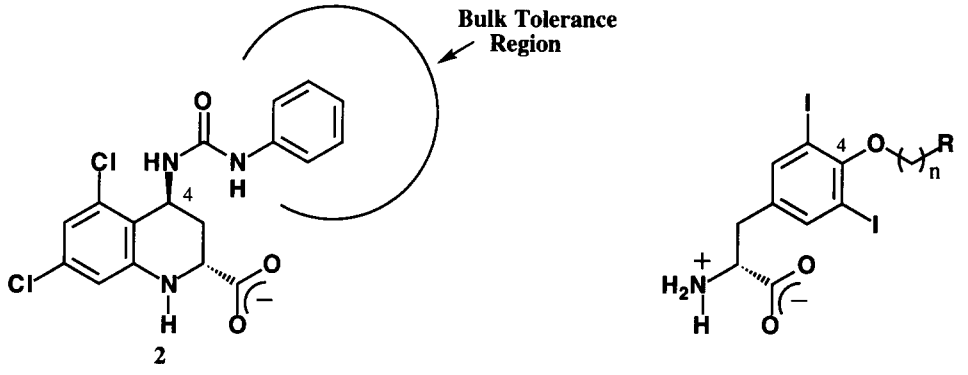


(5,7-Dihalokynurenic acid)



However, this does not take into account the requirement for a hydrophobic substituent on the phenolic oxygen and that 6-substitution in the kynurenic acid series appears to be detrimental to binding affinity.[3] Instead, it is plausible that the diiodophenyl ring, bearing a lipophilic R group, accesses the bulk tolerance region proposed for the 4-substituent of L-689,560 **2**[2,4] (Figure 2).

Figure 2



Summary

We have identified the diiodo-*D*-tyrosine derivative **(*R*)-4** and related analogues as novel NMDA glycine-site antagonists. These aromatic amino acid derivatives comprise a remarkable series of ligands since few α-amino acids display high glycine-site affinity, with activity generally confined to small neutral compounds and affinity decreasing with increasing side-chain size.[2,12] The key structural features for binding are the α-amino acid function, having the (*R*) absolute stereochemistry in common with other glycine-site ligands, and the 3,5-diiodo substituted aromatic group. A second phenyl ring attached at the phenolic oxygen of the tyrosine moiety by a zero to two carbon tether conferred sub-micromolar binding affinity. An interesting finding was that the thyromimetic *D*-thyroxine **12**, the unnatural enantiomer of the thyroid hormone, had equivalent glycine-site affinity to **(*R*)-4**.

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