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An asymmetric approach to the synthesis of a carbon-11 labelled gliotransmitter D-serine

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Abstract Endogenous D-serine, a co-transmitter of glutamate for synaptic *N*-methyl-D-aspartate receptors, is implicated in an array of health conditions. The feasibility of a rapid asymmetric preparation of carbon-11 labelled gliotransmitter D-serine is demonstrated via the hydroxymethylation of a chiral nickel(II) complex. Using an automated radiochemistry synthesiser the key intermediate was obtained with 80 % diastereomeric excess in a 1 min reaction. Further optimisation of the starting glycine synthon is possible in order to achieve even higher stereoselectivity of synthesis, which can benefit subsequent separation–deprotection of the diastereomeric intermediate.

Keywords Amino acids · D-[^{11}C]Serine · Gliotransmitter · Neurotransmitter · Nickel · PET · Schiff bases

Introduction

Amino acids labelled with positron-emitting isotopes were one of the earliest developed PET radiotracers. Some amino acids and their analogues, such as L-[^{11}C]methionine and *O*-(2-^{18}F-fluoroethyl)-L-tyrosine has since then become favoured tracers in oncology. Besides, the development of radiolabelling methods for amino acids using carbon-11 and fluorine-18 remains an important research topic [1–3]. Endogenous D-serine is a co-transmitter of glutamate for synaptic *N*-methyl-D-aspartate receptors (NMDARs). Receptor affinity of NMDAR for binding D-serine versus glycine depends on its GluN2 subunit composition. For the activation of NMDARs glutamate binds to the GluN2 subunit of the receptor and a second ligand binds to the GluN1 subunit. D-Serine is a ligand for the glycine site of the GluN1 subunit receptors in the brain in the case when NMDARs are composed of the GluN1 and the GluN2A subunits. NMDARs composed of the GluN1 and the GluN2B subunits preferentially bind glycine at GluN1 sites [4, 5]. Endogenous D-serine is produced by the epimerisation of L-serine in neurones by serine racemase. Resulting D-serine is transported into astrocites for storage. Na^+-independent alanine–serine–cysteine transporter-1 is found exclusively in neurons, Na^+-dependent ASCT1 and ASCT2 are present in both neurons and astrocites. It was demonstrated that D-serine plays an important role in the formation and maturation of synaptic contacts and in the earlier stages of neuronal circuit construction as a regulator of neuroblast migration in the developing brain. It has been tested as a therapeutic agent for the treatment of schizophrenia, depression, Parkinson disease and post-traumatic stress disorder (PTSD) [6]. Further, D-serine is implicated in stress-related disorders [7], age-related memory loss [8], amyotrophic lateral sclerosis (ALS) [9], apoptosis related to neurotoxins and neurodegenerative disorders [10] among others [6, 11]. Recently, the influence of D-serine to interaction of serotonin 2A receptors with their agonists was described [12]. Contingent on having favourable

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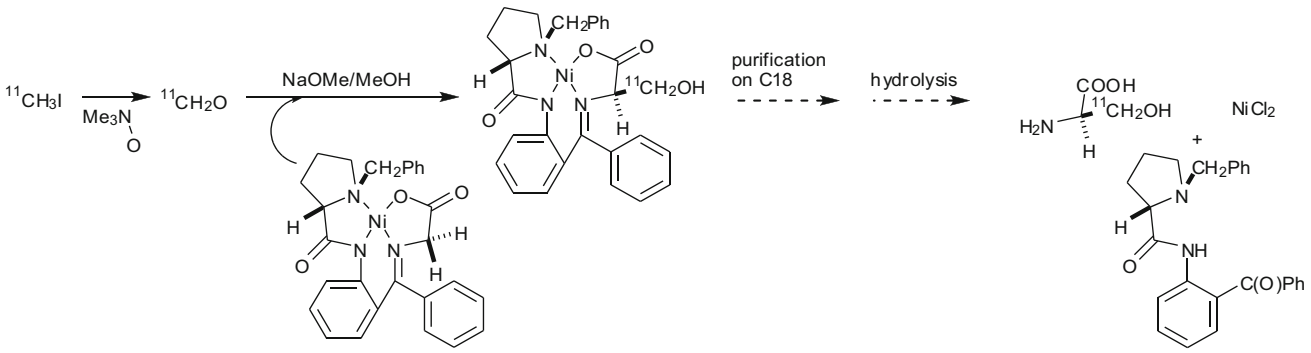

Scheme 1 Preparation of carbon-11 labelled D-serine

pharmacological properties D-serine labelled with ^{11}C may become a useful tracer helping better understand these conditions by non-invasive imaging of the brain using positron emission tomography (PET).

Considering the choice of the radionuclide the isotopic labelling using ^{11}C would be advantageous because the resulting labelled compound will fully retain chemical and biochemical properties. Previously a number of amino acids have been labelled using chemical and biochemical routes. In this communication we report a proof of concept preparation of D-serine intermediate via rapid asymmetric synthesis mediated by a chiral nickel(II) Schiff base complex.

With a growing use of PET in preclinical and clinical settings the tracer synthesis automation has become an important aspect with respect to regulatory (cGMP) and radiation safety guidelines. In particular this is valid for the multi-step synthesis described in this communication.

Experimental

The synthesis was performed using a remote-controlled robotic synthesiser Scansys^{TM} installed in the Cyclotron facility at Herlev University Hospital. The analysis of the reaction mixtures and intermediates was carried out using Shimadzu Prominence HPLC equipped with a diode-array and a γ-ray detectors and Phenomenex Luna C18 5μ, 4.6 × 100 mm column. A linear gradient was run from 30 to 50 % of methanol and water.

Isotopically unmodified reference standards were prepared according to published procedures [13, 14]. Nickel(II) complex of the Schiff base of (*S*)-*N*-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide (BPB) and glycine was prepared according to [15].

[^{11}C]Methyl iodide was obtained via the gas phase iodination of methane. Carbon-11 labelled formaldehyde was prepared by the oxidation of [^{11}C]methyl iodide by trimethylamine oxide as described by Hooker et al. [16].

[^{11}C]Hydroxymethylation of the glycine synthon

A solution of the nickel(II) complex of the Schiff base of BPB and glycine (2 mg, 4 μmol) in 2 M MeONa in methanol (1 ml, 2 mmol) was added to [^{11}C]formaldehyde at 25 °C. In 1 min 5 % aqueous citric acid (5 ml) was quickly added to the reaction mixture and nitrogen was bubbled through the solution for efficient mixing. The reaction mixture was then transferred into a solid-phase extraction module fitted with a C18 cartridge. The cartridge was washed with 20 % aqueous methanol (10 ml) followed by elution of the [^{11}C]hydroxymethylated product with 60 % MeOH in water (10 ml).

Results and discussion

A multi-enzymatic synthesis of carbon-11 labelled L-serine was published in 1990 [17]. For the preparation of D-[^{11}C]serine we employed BPB in nickel(II) complex of its Schiff base with glycine [13, 18, 19]. This glycine synthon enables the creation of desired stereochemistry of the chiral centre of D-serine (Scheme 1). Mass-spectral and NMR properties of such complexes have been studied in great detail [18, 20–22]. The complexes are stable during storage at ambient temperature. Previously, similar synthons were successfully used for the preparation of aromatic α-methyl amino acids labelled with carbon-11 [23].

The [^{11}C]hydroxymethylation leads to the labelled complex of the Schiff base of D-serine with decay corrected radiochemical yield above 50 % based on [^{11}C]methyl iodide (Fig. 1). The peak with the retention time of 6.7 min corresponds to the complex containing D-[^{11}C]serine and the peak with the retention time of 7.2 min corresponds to the complex containing L-[^{11}C]serine, both correlated well with retention times of the respective standards on the UV channel 6.5 and 7.0 min. The first experiments have produced a 80 % diastereomeric excess of the anticipated compound. The mixture of the diastereomers could be

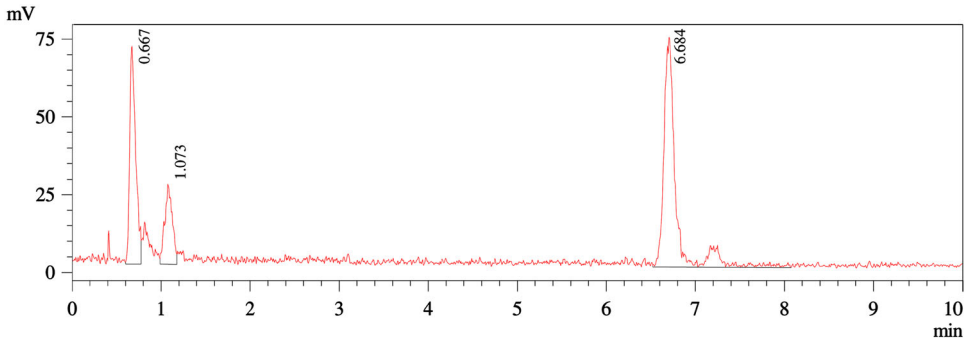

Fig. 1 [^{11}C]Hydroxymethylation. Radiochromatogram of the quenched reaction mixture before the solid-phase extraction step

separated using HPLC. Preliminary optimisation of the separation of the Schiff base of D-serine was performed on C-18 SPE columns and proved feasible.

Further synthesis optimisation is necessary in order to develop a stereospecific synthesis which does not require separation of the diastereomeric intermediates. More stereoselective chiral auxiliaries for hydroxyalkylation of glycine synthons have been described in the literature [24]. Several stereospecific glycine synthons have been developed for use in alkylation reactions [25]. Our intention is to test their performance for the preparation of carbon-11 labelled D-serine.

Conclusions

Feasibility of the efficient and rapid asymmetric preparation of a D-serine intermediate was demonstrated using an automated synthesiser. High diastereomeric excess (80 %) of [^{11}C]hydroxymethylation was achieved starting with nickel(II) complex of the Schiff base of (*S*)-*N*-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide and glycine.

For the convenient clinical application of the labelled gliotransmitter further optimisation of the starting glycine synthon is necessary in order to develop a stereospecific synthesis and avoid separation of diastereomeric intermediates.

Provided high asymmetric induction is achieved during the second step of the process (Scheme. 1) the product should be amenable for automation on radiochemistry synthesisers that do not have LC module as a constituent.

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