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THE LABELLING OF A NOVEL TROPANE DERIVATIVE [^{11}C]NS 2214 (BMS-204756) - AN INHIBITOR OF THE DOPAMINE TRANSPORTER

***A. D. Gee,[1] P. Moldt[2] and A. Gjedde[1]**

[1] *PET Centre, Aarhus University Hospital, Nørrebrogade 44, 8000 Aarhus C., Denmark*

[2] *Neurosearch A/S, 26B Smedeland, 2600 Glostrup, Denmark*

Summary

The ^{11}C-labelling of a novel tropane derivative (+)-*(E)-(1R,2R,3S)*-3-(3,4-dichlorophenyl)-8-[^{11}C]methyl-8-azabicyclo[3.2.1]octane-2-*O-methyl*-aldoxime (NS 2214 or BMS-204756), an inhibitor of the dopamine transporter (IC_{50} 3 nM) is reported. (+)-*(E)-(1R,2R,3S)*-3-(3,4-dichlorophenyl)-8-azabicyclo[3.2.1]octane-2-*O-methyl*-aldoxime (NS 2262, either as the disulphate salt with added base or as the free amine) was alkylated with [^{11}C]methyl iodide. The crude product was purified by HPLC using either a reverse phase or cyanopropyl stationary phase. Best results were obtained using the free amine as the labelling precursor and the cyanopropyl stationary phase for the HPLC purification. This resulted in the synthesis of radiochemically pure [^{11}C]NS 2214 with a decay corrected radiochemical yield of 24 - 30 %, specific activity > 50 GBq / μmol (> 1.4 Ci / μmol), and a total synthesis time of less than 30 min (counted from EOB).

Key words: ^{11}C, dopamine transporter, PET, NS 2214, BMS-20457

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Introduction

In recent years there has been a surge of interest in the use of radiotracers as *in vitro* and *in vivo* probes of the dopamine transporter / reuptake site (DAT). [^{11}C]Nomifensine,[1] the first reported ligand for DAT imaging using positron emission tomography (PET), proved useful in the study of the reuptake site in unilateral MPTP lesioned monkeys, and in Parkinsonian patients.[2-8] Subsequently, a number of benzhydryl piperazine derivatives,[9-14] cocaine,[15,16] cocaine analogs[17-28] and other compounds[29,30] have been evaluated as DAT ligands for PET. The recent success of the cocaine congeners [^{11}C]β-CFT (WIN 35-428)[17] and [^{11}C]β-CIT (RTI-55)[18] in dopamine transporter imaging has led to the development of an astonishing number of PET radioligands (Table 1). Although these ligands have been labelled in various positions with a number of radionuclides (^{11}C, ^{18}F and ^{76}Br), all of them posess the following structural features (see Table 1.):

a) N-alkyl or N-fluoroalkyl substitution in the 8-position

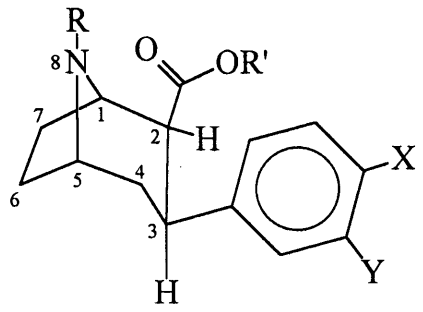
b) alkyl or fluoroalkyl carboxylic esters in the 2-position

c) the 2-carboxylic ester in the exo- (β-) configuration

d) a 3-phenyl group (usually substituted with a halogen in the para position)

e) the 3-phenyl group in the exo- (β-) configuration

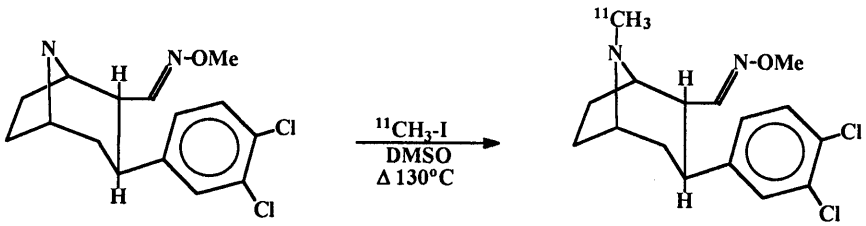
These radioligands are generally characterised by high *in vivo* selectivity, affinity and specific to non-specific binding ratios.



	X	Y	R	R'	Ref
β-CFT (WIN 35,428)	F	H	CH_3	CH_3	17, 19
β-CFT-FE	F	H	CH_2CH_2F	CH_3	20
β-CFT-FP	F	H	$CH_2CH_2CH_2F$	CH_3	20
β-CCT (RTI-131)	Cl	H	CH_3	CH_3	21,22
β-CDCT	Cl	Cl	CH_3	CH_3	22
β-FECT (β-FE-CCT)	Cl	H	CH_3	CH_2CH_2F	21
β-FPCT (β-CCT-FP)	Cl	H	$CH_2CH_2CH_2F$	CH_3	23
β-FIPCT (β-FiP-CCT)	Cl	H	CH_3	$CH(CH_3)CH_2F$	24
β-CBT	Br	H	CH_3	CH_3	25
β-CBT-FE	Br	H	CH_2CH_2F	CH_3	25
β-CBT-FP	Br	H	$CH_2CH_2CH_2F$	CH_3	25
β-CIT (RTI-55)	I	H	CH_3	CH_3	18
β-CIT-FE	I	H	CH_2CH_2F	CH_3	26
β-iP-CIT (RTI-121)	I	H	CH_3	$CH(CH_3)_2$	27
β-CIT-FP	I	H	$CH_2CH_2CH_2F$	CH_3	28
β-CMT (RTI-32)	CH_3	H	CH_3	CH_3	21
β-FETT (β-FE-CMT)	CH_3	H	CH_3	CH_2CH_2F	21

Table 1. The structure of recently reported cocaine analogs labelled with the positron emitting radionuclides ^{11}C, ^{18}F and ^{76}Br. Note that all the above ligands are substituted with a carboxylic ester moiety in the two position with an exo- (β-) configuration, in comparison to NS 2214, Scheme 1.

In parallel with the interest of cocaine analogs as radiotracers, there has been a growing interest in the therapeutic possibilities of DAT blockers for the treatment of neurological disorders. The design of cocaine analogs with high DAT affinity and low toxicity / dependence liability, is however, a formidable task for the pharmaceutical chemist. Cocaine analogs containing 3-carboxylic acid functionalities in the exo- (β-) conformation (Table 1) are known to exhibit powerful stimulant effects, limiting their potential as therapeutic agents. However, recently a new class of cocaine congeners possessing 3-substituted aldoxime prosthetic groups in the endo- (α-) configuration (Scheme 1) have been reported to posess high DAT affinity and a low toxicity / side effect profile.[31]



NS 2262 **NS 2214**

Scheme 1. The labelling of [^{11}C]NS 2214. Note the structutral differences of NS 2214 compared with the ligands shown in Table 1.

Due to these desirable attributes, one such compound, NS 2214 ((+)-*(E)-(1R,2R,3S)*-3-(3,4-dichlorophenyl)-8-[^{11}C]methyl-8-aza-bicyclo[3.2.1]octane-2-*O-methyl*-aldoxime), (IC_{50} 3 nM), is presently under evaluation as a therapeutic agent for the treatment of Parkinson's Disease.

Because of these unique properties, NS 2214 was selected as a candidate for ^{11}C-labelling and *in vivo* evaluation as a PET ligand for the dopamine transporter. This report describes two methods for the synthesis and purification of [^{11}C]NS 2214.

Experimental

General - [^{11}C]CO_2 was prepared by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a nitrogen gas target and 16 MeV protons produced by the GE Medical Systems PETtrace cyclotron at Aarhus University Hospital. Samples of NS 2262 (free amine and sulphate) and NS 2214 sulphate were obtained as gifts from Neurosearch A/S, Glostrup, Denmark.

Hydriodic acid (57 %, unstabilised), sodium hydride (60 % oil dispersion), anhydrous dimethyl sulphoxide (DMSO) and HPLC grade acetonitrile were purchased from Aldrich. Anhydrous tetrahydrofuran (THF) and lithium aluminium hydride (LAH) were obtained from Merck. All above reagents were used without further purification.

LAH (ca. 500 mg portions) was transferred, under argon, to 5 cm^3 vials which were subsequently sealed and stored in a desiccator under argon until required. A fresh saturated (ca. 1 M) solution of LAH was made before each experiment by the addition of ca 5 cm^3 anhydrous THF to the vials containing LAH under an inert atmosphere. The labelling procedure, including preparation of [^{11}C]methyl iodide,[32,33] alkylation, semi-preparative HPLC purification, rotary evaporation, formulation and sterile filtration, was performed using a fully automated system.[34]

Semi-preparative HPLC was performed using a Perkin Elmer model 200 isocratic pump equipped with a 5 cm^3 injection loop and connected in series with a an Applied Biosystems 759A variable wavelength UV detector (λ = 270 nm) and a radiodetector of in-house design. One of the following chromatographic systems were used for the purification of [^{11}C]NS-2214:

System A: Nucleosil C18 column, mobile phase: 0.010 M H_3PO_4 / acetonitrile (3 : 7), flow 10 cm^3 / min, wavelength 225 nm.

System B: Nucleosil 5 CN column, mobile phase: 25 mM NaH_2PO_4 / acetonitrile (2:1), flow 10 cm^3 / min, wavelength 225 nm.

Analytical HPLC was performed using a Perkin Elmer model 250 binary pump equipped with a 20 µl injection loop and connected in series with an Applied Biosystems 759A variable UV detector (λ = 225 nm) and a radiodetector of in-house design. The following column and mobile phase was used: Nucleosil 5 CN column (250 x 4.6 mm, 5 mm), 50 mM NaH_2PO_4 / acetonitrile (2:1), flow 2 cm^3 / min.

[^{11}C]Methyl Iodide - [^{11}C]Carbon dioxide was purged form the target in a stream of nitrogen gas and trapped on 4 Å molecular sieves. On heating, the [^{11}C]O_2 was released and passed through a solution of LAH (300 cm^3). On completion of [^{11}C]O_2 transfer, the THF was evaporated and 1 cm^3 hydriodic acid was added. On heating at 160 °C the [^{11}C]methyl iodide formed was distilled in a stream of nitrogen gas to a reaction vial containing the labelling precursor.[32,33]

[N-methyl-^{11}C]NS 2214 (Scheme 1)

^{11}C-Alkylation of NS 2262 was achieved by two methods, using either NS 2262 disulphate or NS 2262 free amine:

Method 1: using NS 2262 disulphate

1 mg NS 2262 disulphate and 1 mg NaH (50% oil dispersion) were dissolved in 300 µl DMSO in a 800 µl septum-equipped vial.

Method 2: using NS 2262 free amine

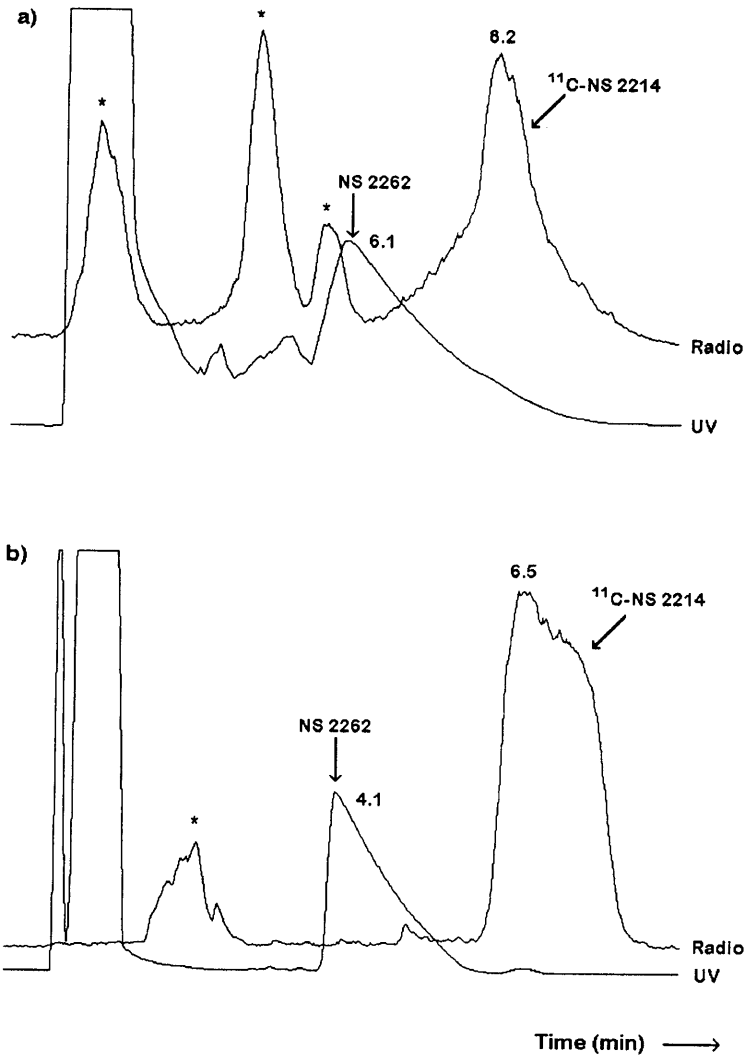
0.5 mg NS 2262 free amine was dissolved in 300 µl DMSO and in a 800 µl septum-equipped vial.

[^{11}C]Methyl iodide was trapped in a solution corresponding to method 1 or 2. After heating for 5 min at 130 °C, the crude product was diluted with 0.5 cm^3 of HPLC mobile phase and purified using either chromatographic system A (C 18 column) or B (5 CN column).

The fraction corresponding to [^{11}C]NS 2214 was collected, evaporated under reduced pressure, formulated in buffered saline and passed over a 0.22 µm filter into a sterile vial.

Results and Discussion

Radiochemically pure (>98%) [^{11}C]NS 2214 was produced by the N-alkylation of NS 2262 with [^{11}C]methyl iodide (Scheme 2) followed by semi-preparative HPLC purification and formulation. The final product was synthesised in less than 30 min (counted from EOB).



Scheme 2. Semi-preparative HPLC chromatograms obtained from the purification of [^{11}C]NS 2214 **a)** using chromatographic system A[#] and labelling method 1[¤], and **b)** using Chromatographic system B[##] and labelling method 2[¤¤]. An asterix (*) denotes an unidentified radiolabelled impurity.

[#] C 18 column, [##] Cyanopropyl (5 CN) column, [¤] Using NS 2262 disulphate salt as precursor + base, [¤¤] Using NS 2262 free amine as precursor

The labelling precursor, NS 2262, was used either as the disulphate salt with added base or as the free amine.

Labelling method 1 (using NS 2262 disulphate):

Using this method only 40 - 50 % of the total radioactivity in the crude product consisted of [^{11}C]NS 2214 (see Scheme 2a). The presence of unidentified radiolabelled impurities suggested that *in situ* generation of the NS 2262 free amine was accompanied by a degree of base induced decomposition of the precursor at the temperatures used (130 °C). The base catalysed isomerisation of the 2-aldoxime moiety from the trans (*E*) to the cis (*Z*) isomer is a likely mechanism of labelled byproduct formation. The purifed product, however, showed only the presence of the trans (*E*) isomer.

Labelling method 2 (using NS 2262 free amine):

In attempts to decrease the presence of ^{11}C-labelled decomposition products in the crude product, the free amine of NS 2262 was used as the labelling precursor. Under identical labelling conditions (except for the omission of NaH), the presence of labelled impurities in the crude product were reduced to 5 - 15 % (see Scheme 2b). Furthermore, the amount of labelling precursor could be reduced (*ca.* 0.5 mg *vs.* 1 mg for the disulphate salt) with no apparent effect on the overall labelling yield. No further optimisation of the labelling conditions was performed.

The purification of crude [^{11}C]NS 2214 was first attempted using a Nucleosil C18 semi-preparative HPLC column eluted with phosphate buffer / acetonitrile (system A). Separation of the [^{11}C]NS 2214 from NS 2262 was made difficult due to significant

peak tailing of these compounds under the chromatographic conditions used (Scheme 2a). This resulted in a content of 200 - 300 μg NS 2262 in the final product. As NS 2262 is itself a potent inhibitor of the dopamine transporter (IC_{50} 2 nm) (34), contamination of the labelled product with the precursor would effectively reduce the 'specific activity' of the [^{11}C]NS 2214. Precursor contamination could be reduced to 50 - 100 μg by collecting the second half of the fraction corresponding to [^{11}C]NS 2214.

With the aim of further reducing the NS 2262 contamination in the final product, an alternative purification system was evaluated. Using a 5-CN semi-preparative HPLC column, eluted with phosphate buffer / acetonitrile (chromatographic system B), peak tailing was significantly reduced (Scheme 2b). Using this method, no NS 2262 was detected in the final product. The specific activity of the [^{11}C]NS 2214 thus obtained was > 50 GBq / μmol (> 1.4 Ci / μmol) at EOS.

Neither of the purification methods used, showed contamination of the final product with the cis (*Z*) 2-aldoxime isomer of [^{11}C]NS 2214.

Conclusions

[^{11}C]NS 2214 can be reproducibly synthesised by the N-alkylation of NS 2262 with [^{11}C]methyl iodide and in sufficient quantities for *in vivo* receptor imaging using PET. Of the methods tested, best results were obtained using NS 2262 free amine as the labelling precursor (labelling method 2) in combination with an HPLC purification step using a cyanopropyl stationary phase (chromatographic system B). Using the above

procedure, radiochemically pure [^{11}C]NS 2214 was synthesised with a radiochemical yield of 24 - 30 % (decay corrected to EOB), specific activity > 50 GBq / μmol (> 1.4 Ci / μmol) at EOS, and a total synthesis time of less than 30 min (counted from EOB). PET studies of the *in vivo* kinetics and binding of [^{11}C]NS 2214 are currently in progress.

Acknowledgments

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