1. Introduction
Dementia is a leading cause of death and disability worldwide. According to Lipton (2006:160) Alzheimer’s disease, which is the leading cause of dementia, ranks fourth in mortality in the United States, followed not so far behind by vascular dementia (multi-infarct dementia). It is clear that neurodegenerative disorders, such as Alzheimer’s disease, Parkinson’s disease and Amyotrophic lateral sclerosis have a devastating impact on not only the patients suffering from the disease, but also on their families.

Glutamate is the single, most powerful neurotransmitter on which the nervous system’s capacity to rapidly convey sensory information and its ability to form thoughts and memories is dependant. Because glutamate is so powerful, its presence in large amounts, or for long periods, can literally excite cells to death, a phenomenon known as excitotoxicity (Lipton, 2006:162). During this excitotoxicity excessive Ca\textsuperscript{2+} influx into the neuronal cell can trigger a series of intracellular mechanisms, leading to apoptosis and neuronal cell death (Figure 1).

Glutamate-related excitotoxic neuronal cell injury and death has been reported to contribute to almost every major neurodegenerative disorder. This mechanism can also harm oligodentrocytes, which provides the myelin sheaths of axons. This means that excitotoxicity can cause white matter (glial) as well as grey matter (neuronal) disorders. One of the ways excitotoxicity can occur is by over activation of N-methyl-D-aspartate-sensitive glutamate receptors and the subsequent excessive Ca\textsuperscript{2+} influx into the associated cell.

2. Aim of study
The aim of the study was to obtain compounds to halt, or at least slow down the rate of excitotoxicity. This was achieved by the synthesis of various novel pentacycloundecylamines, selected because of their well documented calcium channel activity and the fact that they are open channel blockers (Van der Schyf et al., 1988:448).

To augment the potential NMDA (N-methyl-D-aspartate) receptor antagonistic effects of the pentacycloundecylamines, each compound incorporated a nitrogen monoxide donating moiety, to S-nitrosylate the crucial cysteine residues in the NMDA receptor, thus leading to receptor desensitisation. The pentacycloundecylamines therefore also served as a scaffold to optimise NO delivery to the target receptor’s cysteine residues, as systemic NO delivery will cause adverse effects associated with vasodilatation. The effect of these compounds on cysteine residues were evaluated by means of a fluorescent assay.

3. Compounds synthesised in this study
Based on the literature, it was decided to synthesise two different types of nitrogen monoxide donating compounds. The first three compounds were unsaturated nitro compounds (Figure 2), and the second group contained three nitro esters, or nitrates (Figure 3). All of the compounds complied with the structural requirements necessary for NMDA antagonism.

4. Synthesis
Starting with Cookson’s diketone, the pentacycloundecylamines was synthesised by means of reductive amination using 2-aminoethanol, except in the cases of compounds 2 and 6. In the case of compound 2 the amine was 3-aminopropanol. The oxa-ether was formed by using NaBH\textsubscript{4} as reducing agent (Figure 4).
The second step in the synthesis entailed the formation of esters utilising activation chemistry with EDC as activating reagent (Figure 5).

For the final step in the synthesis of compounds 4, 5 and 6, their respective hydroxyl groups were nitrated using thionylchloride nitrate (equation 2). The thionylchloride nitrate was prepared using equation 1 below (Hakimelahi et al., 1984:907).

\[
\text{AgNO}_3 + \text{SOCl}_2 \leftrightarrow \text{AgCl} + \text{SOClNO}_3 \quad \text{-(1)}
\]

\[
\text{SOClNO}_3 + \text{ROH} \leftrightarrow \text{RONO}_2 + \text{SOCl(OH)} \quad \text{-(2)}
\]

The synthesis of compound 6 was carried out by means of the catalytic reduction of NGP1-01 with palladised carbon. The resulting pentacycloundecylamine was then reacted with 4-hydroxybenzaldehyde and dehydrated under Dean-Stark conditions to give the intermediate alcohol compound (Figure 6).

5. S-nitrosylation assay

The nitrogen monoxide donating compounds (4mM of each) were added to an excess cysteine, dissolved in HEN buffer (pH 7.7) and stirred at room temperature for 1 hour. In the next step the unreacted thiols were blocked with the thiol-specific methylthiolating agent, methylmethaneithiosulfonate (MMTS). MMTS doesn't react with pre-existing disulphide bonds or nitrosothiols. The mixture was stirred for another 20 minutes at 50 °C, after which acetone was added to precipitate the unreacted MMTS. The mixture was filtrated and N-[6-(biotinamido)hexyl]-3’-(2’-pyridyldithio)propionamide (biotin-HPDP), a sulphydryl-specific biotinylation reagent, was added to the filtrate. Upon reaction with cysteine, the biotin-HPDP splits of pyridine-2-thione, which absorbs UV radiation at 343 nm.

The UV absorbance was measured at different points in time over 20 minutes, and the intensity of the absorbance was an indication of how much biotin-HPDP had reacted. The amount of biotin that reacted is directly proportional to the extent to which nitrosylation took place (Figure 7).
6. Results
As can be seen from Figure 8, the nitrate compounds showed much higher nitrosylating (and therefore nitrogen monoxide donating capability) than the unsaturated nitro compounds. This is to be expected, since most of the drugs on the market that are used for their nitrogen monoxide donating effects are nitrates, such as nitroglycerine. It has also become clear that an electron withdrawing group, conjugated to the nitrogen monoxide donating moiety increases its nitrogen monoxide donating potential.

7. Conclusion
The novel compounds synthesised in this study are now proven nitrosylators and comply with the structural requirements for NMDA receptor antagonism. It can therefore be postulated that they show promise as potential drug leads in the treatment of neurodegenerative diseases. A calcium flux assay is currently underway to unambiguously prove whether or not S-nitrosylation does in fact augment the calcium channel activity of polycyclic cage amines.

References:

The LENGTHth and breadth of academia

Pharmacy, being a labour and knowledge intensive profession, requires considerable concentration when functions need to be performed. With our noses always to the grindstone, we seldom step back to reflect on the diversity within our profession and consequently, the many roles pharmacists need to fulfil to satisfy societal health needs. We all know of the shortage of conventional pharmacists in hospitals and community practices. However, there are certain specialist areas where the need is even greater. One such area is radio- or nuclear pharmacy. Currently, we only have two registered radiopharmacists in South Africa. Yet, measured by world standards, we have a fairly significant nuclear medicine industry. The shortage of radiopharmacists is a worldwide phenomenon but is particularly acute in developing countries. In most countries radiopharmaceuticals are manufactured and in some even administered to patients by physicists and chemists. Although one cannot dispute their knowledge of nuclear physics and radiation chemistry, it is doubtful that they have a sufficient understanding of GMP requirements and aseptic technique, let alone patient care.

In November of last year, I was fortunate to be invited to the International Atomic Energy Agency (IAEA) in Vienna to take part in a consultative meeting with experts in nuclear medicine. The purpose of the meeting was to develop a curriculum for the training of qualified persons to manufacture and release/dispense radiopharmaceutical medicines. The term qualified person is used since the programme is aimed at all persons, not only pharmacists, who are employed in radiopharmaceutical manufacturing facilities. The programme is intended to be offered in South Africa in conjunction with a pharmacy school once formally approved by the IAEA and will be open to international as well as local candidates. This is an exciting development and it is hoped that many young pharmacists will make use of this opportunity to specialise in this underdeveloped area in pharmacy. Accreditation by the Pharmacy Council will be sought once the programme is ready for implementation.