Summary

Parkinson’s disease is a serious neurological disorder of the central nervous system that usually becomes apparent after the age of 55. It concerns the increased deterioration of neurons responsible for the normal functioning of the human motor system. This increased deterioration of neurons starts long before the symptoms become apparent which is after about $\frac{3}{4}$ of the dopaminergic neurons that project to the striatum are lost. Today is it possible to assess an increased loss of dopaminergic neurons in an early stage by conducting smell tests. However, an early diagnosis does not mean that thereafter the development of Parkinson’s disease can be prevented or slowed down. At present, there is no known cure for Parkinson’s disease, yet the apparent symptoms can be controlled.

Because Parkinson’s disease is associated with the loss of dopaminergic nigrostriatal neurons, a shortage of the neurotransmitter dopamine arises in the striatum. This neurotransmitter is responsible for a normal functioning of the human motor system. Dopamine is involved in the fine-tuning of the conscious movement of muscles. Theoretically, a shortage of dopamine could be compensated by administration of extra dopamine, yet dopamine is unable to penetrate the brain after peripheral administration, and intracranial administration is considered a too invasive method. Treatment of the symptoms of Parkinson’s disease therefore is limited to the administration of drugs that after transport to the brain are converted to dopamine and centrally acting drugs that directly or indirectly mimic the actions of dopamine.

Early stage parkinsonism can be treated by the administration of anti-cholinergics. Slowing the cholinergic activity in the striatum reinstates the balance between activation and inhibition of deliberate movement. As the disease progresses and more dopaminergic neurons have deteriorated, the symptoms can be treated with dopamine agonists. Though dopamine agonist treatment in general seems an effective treatment, these drugs give very different results in Parkinson’s disease patients. Once the disease has progressed to an advanced state, treatment with L-dopa is considered. L-dopa has the highest efficacy of all anti-parkinsonian drugs available, yet it is most vulnerable to metabolism that it can only be effective when co-administered with metabolism inhibitors. L-dopa is administered in relatively high dosages after which it is actively transported to the brain where it is converted to dopamine. L-dopa therapy is accompanied by serious adverse events that can be suppressed by co-medication. Further suppression of L-dopa metabolism, allows for lower dosages but because of non-specific
metabolism inhibition, this approach causes other adverse effects. The dosage of L-dopa can be brought down further by co-medication of dopamine agonists.

It is important that L-dopa therapy is started only when all other available drugs have lost their efficacy. L-dopa is responsible for an increasingly rapid deterioration of dopaminergic neurons and, a large proportion of the patients is confronted with serious complications. After a few years the efficacy of L-dopa therapy wears off and at that point no other drugs are available that can successfully alleviate parkinsonian symptoms. Only the mixed D₁/D₂ agonist, apomorphine, is able to improve the condition of Parkinson’s disease patients for a short time. Together with L-dopa, apomorphine has the highest clinical efficacy reported in the treatment of Parkinson’s disease. Its application is limited because, like L-dopa, it is prone to rapid metabolism and cannot be orally administered. The recent positive clinical results and recent developments in the area of drug delivery seem to bring a change. The discovery that apomorphine is able to induce regeneration of dopaminergic neurons further propels the interest for this drug. Research dedicated to develop drugs that are able to induce proliferation of existing neurons and protect them has enjoyed increasing interest over the last years.

The two most efficacious drugs for the treatment of Parkinson’s disease belong to the family of catecholamines with affinity for both the dopamine D₁ and D₂ receptor. In the 70’s and 80’s, already many chemical structures emerged out of the medicinal chemistry laboratories. Based on the structural resemblance of dopamine and apomorphine many analogs were synthesized that provided great insight in the 3D-constitution of the active site of the dopamine receptor. Despite the discovery of many potent dopaminergic catecholamines, none of these compounds were ever developed to a drug for the treatment of Parkinson’s disease. The large susceptibility for metabolism, suspected toxicity, and the availability of promising other types of dopamine agonists caused science to divert its attention away from catecholamines.

Prodrugs are, like L-dopa, biologically inactive chemical structures that are being bioactivated by the body to a biologically active metabolite. A prodrug, in general, is designed in such a way that it is less or not susceptible for metabolism than the active metabolite, the actual drug. Pharmacological relevant concentrations of the active metabolite should build up soon after administration of the prodrug. Ideally this would have to be followed by a continuous bioactivation of the prodrug to keep steady and effective blood levels of the active metabolite for as long as possible. In general, a lot of ester, amide, and carbamate derivatives of catecholamines were synthesized and some were successfully evaluated in animal model of
Parkinson’s disease. The prodrug strategy so far has not yielded a drug on the marked because they showed little or no improvement over the available alternatives. To date the only prodrug that is applied to treat a central nervous system disorder is L-dopa.

This thesis describes the research that was conducted after the unsuspected observation that a biologically inactive chemical compound, an enone (PD148903), by unknown process proved to induce dopaminergic stereotypy in an animal model. Further investigation had indicated that the inactive compound was being bioactivated to a dopamine agonist in the body of rat.

**Chapter 2** is an in depth investigation. It became evident that PD148903 is orally active and is bioactivated to one or more compounds with affinity for the dopamine D₁ and D₂ receptors. After separation and pharmacological evaluation of the enantiomers of PD148903, the absolute configuration was established, proving the (S)-enantiomer (PD217015) to be responsible for the pharmacological effect. Both PD148903 and PD217015 were able to dose-dependently alleviate parkinsonian symptoms in an animal model of Parkinson’s disease. PD217015 is effective in this model even at low doses and is being converted to a known dopaminergic catecholamine, (S)-5,6-di-OH-DPAT. This active metabolite is found in blood serum and in the brain. The (R)-enantiomer of this prodrug, PD217016, is converted to (R)-5,6-di-OH-DPAT that could only be detected in blood serum. With these findings, a fundamentally new prodrug strategy has been discovered with the potential of revolutionizing the treatment of Parkinson’s disease with orally active prodrugs of dopaminergic catecholamines.

**Chapter 3** describes the research that followed to investigate the generality of the *in vivo* enone bioactivation to catecholamines. Through the synthesis and pharmacological evaluation of a number of compounds analogous to PD148903 of which the expected active metabolites would have potential application in the treatment of Parkinson’s disease it was learned that bioactivation was not restricted to PD148903 alone. In fact, most of the tested compound showed to be efficacious in the Ungerstedt rat model for Parkinson’s disease. From data obtained in this model, it was clear that one of the analogous (racemic) compounds induced a significantly stronger pharmacological effect than PD148903 did. After administration of these two compounds at high dosages in separate rats, analysis of brain tissue showed the presence of a new metabolite. Interpretation of mass-spectra of these compounds has lead to a hypothesis on how enones were bioactivated to their corresponding catecholamines. In the hypothesis it is
supposed that an enone first is hydroxylated next to the ketone after which oxidation and keto-enol tautomerization lead to the corresponding catecholamine.

**Chapter 4** deals with an essential part of prodrug design. Because PD217015 and the *in vivo* active analogs are being bioactivated at great speed, there is a possibility that slowing down this bioactivation will prolong the duration of action and stabilize the pharmacological effect. This has great advantages for treatment because it then can take place less frequently and there is a constant therapeutic effect. The approach chosen to achieve this goal was by the synthesis of cascade prodrugs. Two series of oxime derivatives of 148903 and PD217015 were synthesized and tested. These cascade prodrugs are to be converted in vivo to PD148903 or PD217015 to subsequently be bioactivated to the pharmacologically active principle. Most of the oximes tested proved to alleviate parkinsonian symptoms in the Ungerstedt rat model of Parkinson’s disease. No clear-cut relation was observed between the structure of the oxime and the pharmacological effect. Large individual differences between the animals tested were detrimental to deriving a structure-activity-relationship. Though at the same dose, oxime derivatives are less efficacious than PD148903 en PD217015, we have shown that oxime derivatives can serve as cascade prodrugs.

**Chapter 5** describes the most efficacious enone prodrug synthesized and tested. Compound (−)-GMC6650 is structurally close to PD217015 yet consists of a three-ring system derived from the known mixed dopamine D₁/D₂ agonist, 6,7-dihydroxy-N-n-propylbenzo[g]quinoline, structurally close to the aporphines. In microdialysis experiments (−)-GMC6650 induced long-lasting dopaminergic effects at very low doses (1 nmol kg⁻¹ (*sc*) 3 nmol kg⁻¹ (*po*), respectively 0.25 en 0.74 µg kg⁻¹). In the Ungerstedt rat model of Parkinson’s disease (−)-GMC6650 was able to alleviate parkinsonian symptoms with a slower onset of action relative to PD217015 was observed. At similar dosage of (−)-GMC6650 and PD217015, (−)-GMC6650 is more efficacious and its duration of action is longer. (−)-GMC6650 seems to be a promising drug candidate for further development.

**Chapter 6** an overview is given of the synthesis of many other enones that have not been extensively tested yet. While Chapter 5 deals with structural, in this chapter a number of structurally more flexible enones is described. Less rigid enone structures could possibly have an influence on the bioactivation mechanism. A number of analogs of PD148903 with the ketone moiety in a different position were synthesized in order to obtain a series of compounds in which the ketone was situated at all different positions around the original enone ring. The
corresponding catecholamines would next to their dopaminergic activity also include serotonergic activity, while other may be partial agonists or antagonists at the dopamine receptor. Bioactivation of these compounds would allow the enone prodrug concept to be applicable for a number of neurological/psychiatric disorders.

This thesis as a whole describes a successful research project that led to the discovery of a fundamentally new prodrug concept. Through sometimes uncommon chemical reactions a number of interesting compounds were prepared with potential applications for the treatment of Parkinson’s disease. More research is necessary to elucidate the bioactivation mechanism of these enone prodrugs in detail.