Bundgaard
Editor

design of prodrugs

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Edited by

Hans Bundgaard

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Preface

During the last decade it has become more obvious that the commonly used processes of delivering therapeutic agents to the sites of their action within the body are generally inefficient and unreliable. Optimization of drug delivery and, consequently, improvement in drug efficacy implies an efficient and selective delivery and transport of a drug substance to its site of action. Recognition of the importance of drug delivery for the therapeutic indices of many types of drugs has been followed by a large increase in research activities in this area, and much attention has been focussed on approaches which aim at enhancing the efficacy and reducing the toxicity and unwanted effects of drugs by controlling their absorption, blood levels, metabolism, distribution and cellular uptake.

Prodrug design comprises an area of drug research that is concerned with the optimization of drug delivery. A prodrug is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation within the body in order to release the active drug, and that has improved delivery properties over the parent drug molecule.

A molecule with optimal structural configuration and physicochemical properties for eliciting the desired therapeutic response at its target site does not necessarily possess the best molecular form and properties for its delivery to its point of ultimate action. Usually, only a minor fraction of doses administered reaches the target area and, since most agents interact with non-target sites as well, an inefficient delivery may result in undesirable side effects. This fact of differences in transport and in situ effect characteristics for many drug molecules is the basic reason why bioreversible chemical derivatization of drugs, i.e., prodrug formation, is a means by which a substantial improvement in the overall efficacy of drugs can often be achieved.

Prodrug research matured as a branch of pharmaceutical research during the 1970s. Over the past decade this chemical approach to optimization of drug delivery has undergone considerable expansion, largely as a result of an increased awareness and understanding of the physicochemical factors that affect the efficacy of drug delivery and action. Several drugs are now used clinically in the form of prodrugs, and as the prodrug approach is becoming an integral part of the new drug design

process one may expect that the new drugs in many cases will appear as prodrugs.

The purpose of the present book is to provide a comprehensive and basic source of information on the recent developments within the prodrug area and on the rational basis for prodrug design. Admittedly, there are numerous review articles and a few texts devoted to one or more topics in the prodrug area, but a current and comprehensive treatment appears to be lacking.

The book is divided into eleven chapters, each written by active scientists in the field. The first chapter provides a review and classification of bioreversible derivatives for various functional groups and chemical entities occurring in drug substances. For most examples discussed due attention is given to the potential therapeutic benefits achievable by the derivatization, e.g., improved absorption. In chapter 2 the design of prodrugs through consideration of enzyme-substrate specificities is discussed. Various enzyme classes are considered and their usefulness as prodrug reconversion sites is discussed in detail.

The ideal site and rate for drug release from a prodrug depend upon the specific delivery problems which are meant to be overcome by the prodrug design. Therefore, the pharmacokinetic aspects are of great importance in prodrug design. Chapter 3 is devoted to these aspects and it provides surveys of the theory accompanying each goal achievable with a prodrug, methods for evaluating the success of the prodrugs and the practical limitations as evidenced by several examples, their successes and failures. Chapter 4 describes the use of the prodrug approach for the development of agents with prolonged duration of activity, including a discussion of polymeric prodrug sustained release delivery systems.

Chapter 5 deals with the very important area of providing site-specific delivery or targeting of drugs to their site of action by the prodrug approach. Several examples of site-specific delivery based on site-specific transport or prodrug cleavage are given, and the importance of the physicochemical properties of the parent drug to the site-specific delivery of drugs via prodrugs is stressed. The use of the prodrug approach to increase the therapeutic index of a drug is considered further in Chapter 6. An extensive review is given, with most examples being taken from amongst steroidal and non-steroidal anti-inflammatory agents, β-stimulants and anticancer agents.

The prodrug approach has been used frequently to solve pharmaceutical formulation problems, such as stability and solubilization. Chapter 7 treats this area of prodrug application in a physicochemical manner, with illustrative examples. In recent years the application of prodrugs to enhance the percutaneous absorption of drugs has received much interest, and this theme is treated in chapter 8. Besides reviewing the previous studies in the field, the chapter provides a rational basis for the design of prodrugs aiming at improving the delivery of drugs to the skin.

Chapter 9 is concerned with anticancer prodrugs. The emphasis is put on prodrugs which improve the pharmacokinetic properties of anticancer agents, and in particular prodrugs which are activated selectively in tumour cells to the active drug.

The design and utility of macromolecular prodrugs is a relatively new area which certainly is going to be the focus of intense research in the near future. In chapter 10 the use of albumin as a transport group or carrier for drugs and, in particular, enzymes is specifically discussed. The promising properties of such conjugates for drug targeting are discussed, as are the many pitfalls and possible disadvantages that any given system might have.

The final chapter (Ch. 11) on prodrugs versus soft drugs has been included in the book in order to clarify some common confusion about these two rather different terms. Whereas a prodrug is an inactive derivative which is activated predictably in vivo to the active drug, a soft drug is an active species like any other drug, but it is designed in such a way that it will undergo a predictable transformation or metabolism to an inactive metabolite. Thus, the common feature of prodrugs and soft drugs is only that a transformation in vivo is involved, it being either an activation (prodrugs) or an inactivation (soft drugs). By definition, the two terms are just opposite to each other. Several examples are given to illustrate the difference between prodrugs and soft drugs, but examples of prodrugs of soft drugs are also included.

This book presents the basic principles of prodrug design and illustrates these principles with many examples. In addition, it provides a comprehensive review of the most recent literature concerning the design and application of prodrugs. Hopefully, the book will be useful to all those concerned with drug delivery and drug design in universities or industry and will initiate new research for increased practical utilization of the prodrug concept.

Hans Bundgaard

Contents

Design of Prodrugs (Bundgaard, H., ed.)

CHAPTER 1

Design of prodrugs: Bioreversible derivatives for various functional groups and chemical entities

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1. Introduction

A basal requisite for the prodrug approach to be useful in solving drug delivery problems is the ready availability of chemical derivative types satisfying the prodrug requirements, the most prominent of these being reconversion of the prodrug to the parent drug in vivo. This prodrug – drug conversion may take place before absorption (e.g., in the gastrointestinal tract), during absorption, after absorption or at the specific site of drug action in the body, all dependent upon the specific goal for which the prodrug is designed. Ideally, the prodrug should be converted to the drug as soon as the goal is achieved. The prodrug per se is an inactive species, and therefore, once its job is completed, intact prodrug represents unavailable drug. For example, prodrugs designed to overcome solubility problems in formulating intravenous injection solutions should preferably be converted immediately to drug following injection so that the concentration of circulating prodrug would rapidly become insignificant in relation to that of the active drug. Conversely, if the objective of the prodrug is to produce a sustained drug action through rate-limiting prodrug conversion the rate of the conversion should not be too high.

The necessary conversion or activation of prodrugs to the parent drug molecules in the body can take place by a variety of reactions. The most common prodrugs are those requiring a hydrolytic cleavage mediated by enzymic catalysis. Active drug species containing hydroxyl or carboxyl groups can often be converted to prodrug esters from which the active forms are regenerated by esterases within the body, e.g., in the blood. In other cases, active drug substances are regenerated from their prodrugs by biochemical reductive or oxidative processes. Sulindac, for example, is active only when reduced to its thioether form [1,2] and a prodrug of the pyridinium

quaternary compound, 2-PAM, is converted to the parent drug through an enzymatic oxidation process in the body [3 – 5]. Besides usage of the various enzyme systems of the body to carry out the necessary activation of prodrugs, the buffered and relatively constant value of the physiological pH (7.4) may be useful in triggering the release of a drug from a prodrug. In these cases, the prodrugs are characterized by a high degree of chemical lability at pH 7.4 while preferably exhibiting a higher stability at, for example, pH 3 – 4. As will be discussed below, examples of such prodrugs include *N*-Mannich bases and various ring-opened derivatives of cyclic drugs. A serious drawback of prodrugs requiring chemical (non-enzymic) release of the active drug is the inherent lability of the compounds, raising some stability-formulation problems, at least in cases of solution preparations. As will be shown later, such problems have, in particular cases, been overcome by using a more sophisticated approach involving pro-prodrugs or cascade latentiation, where use is made of an enzymatic release mechanism prior to the spontaneous reaction.

Several types of bioreversible derivatives have been exploited for utilization in designing prodrugs. The purpose of the present chapter is to discuss various chemical approaches to obtain prodrug forms, with due attention to the potential therapeutic benefits achievable by the prodrug approach and with emphasis on recently developed types of bioreversible derivatives. In the past, esters mostly have been considered as prodrug types, and the best known prodrugs are in fact esters of drugs containing hydroxyl or carboxyl groups. Various reviews [6, 7] have dealt

TABLE 1
Examples of Ester Derivatives Developed as Prodrugs for Drugs Containing a Carboxyl Group

Drug	Ester	Reference
Prostaglandins	Phenyl esters	39, 40
γ-Aminobutyric acid	Aliphatic and steroid esters	41, 42, 43
Acetylsalicylic acid	Methylsulphinylmethyl ester	44,45
	Triglycerides	46, 47
L-Dopa	Methyl ester	48
Niflumic acid	β-Morpholinoethyl ester	49
Non-steroidal anti-inflammatory drugs	Methyl esters	50
Amino acids	Glycolic and lactic acid esters	51
Carbenicillin	Aliphatic and aromatic esters	52
Ibuprofen	Guiacol ester	53
Indomethacin	Triglycerides	54, 55
	Phenyl esters	56
	Glycolic acid ester	57
Glutathione	Ethyl ester	57a
Nicotinic acid	Tetrapentaerythritol ester	58, 58a

with esters as prodrug types, and therefore this important class will only be briefly treated herein.

Some other reviews have, more or less specifically, dealt with various prodrug types [8 – 13] and/or paid much attention to enzyme systems available in the organism and their utilization for performing the necessary conversion of prodrugs [14 – 17]. Furthermore, much information on the subject can be gathered from reviews dealing with several other aspects of prodrugs [18 – 24]; see also other chapters in this book.

2. Esters as prodrugs for compounds containing carboxyl and hydroxyl groups

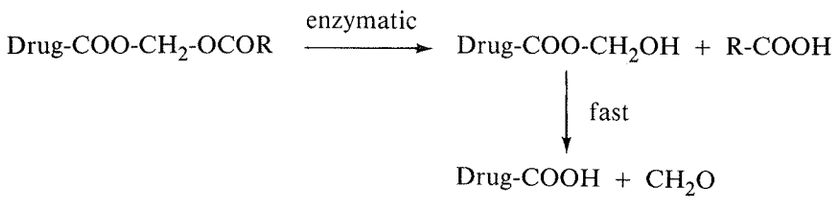
The popularity of using esters as a prodrug type for drugs containing carboxyl or hydroxyl functions (or thiol groups) stems primarily from the fact that the organism

TABLE 2
Examples of Ester Derivatives Developed as Prodrugs for Drugs Containing a Hydroxyl Group

Drug	Ester	Reference
Salicylic acid	Carboxylate and carbonate esters	59, 60
Paracetamol	Carbonate esters	61, 62
	Phosphate ester	63
Trichloroethanol	Carbonate esters	64, 65
	Phosphate ester	66
Cymarol	Diacetylester	67
Vidarabine	Mono- and diesters	68, 69
	Phosphate ester	70
Thymidine	Pivaloate	71
Oxazepam, lorazepam	Aliphatic and aromatic esters	72 – 74
	Amino acid esters	75
Metronidazole	Aromatic esters	76 – 78
	Phosphate ester	79
	Amino acid esters	80, 81
Chloramphenicol	Palmitate and hemisuccinate	82
Various steroids	Various esters	19
Phenols	Amino acid esters	83
Lincomycin	Dialkylcarbonate esters	84
Epinephrine	Dipivaloate	85
Etilefrine	Aliphatic and aromatic esters	86
2-Amino-6,7-dihydroxytetrahydronaphthalene (6,7-ADTN)	Various diesters	87
Terbutaline	Mono- and diesters	88
Isoproterenol	Ditoluyl and dipivaloyl esters	89
Cytarabine	Various mono- and diesters	90
Digitoxigenin	Amino acid esters	91
Acyclovir	Amino acid and hemisuccinate esters	92

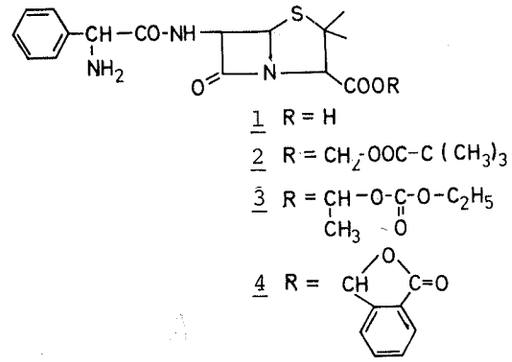
is rich in enzymes capable of hydrolyzing esters. The distribution of esterases is ubiquitous, and several types can be found in the blood, liver and other organs or tissues. In addition, by appropriate esterification of molecules containing a hydroxyl or carboxyl group it is feasible to obtain derivatives with almost any desirable hydrophilicity or lipophilicity as well as in vivo lability, the latter being dictated by electronic and steric factors. Accordingly, a great number of alcoholic or carboxylic acid drugs have been modified for a multitude of reasons using the ester prodrug approach. Several examples can be found in various reviews [6, 7, 14, 18–20] and in Tables 1 and 2.

Sometimes, simple aliphatic or aromatic esters may not be sufficiently labile in vivo to ensure a sufficiently high rate and extent of prodrug conversion. This is the case with penicillin esters. Although various simple alkyl and aryl esters of the thiazolidine carboxyl group are hydrolyzed rapidly to the free penicillin acid in animals, such as rodents, they proved to be far too stable in man to have any therapeutic potential [25]. This illustrates also – as do many other examples – the occurrence of marked species differences in the in vivo hydrolysis of ester prodrugs. A solution to the problem was found in 1965 by Jansen and Russell [26], who showed that a special double ester type (acyloxymethyl ester) of benzylpenicillin was hydrolyzed rapidly in the blood and tissues of several species, including man. The first step in the hydrolysis of such an ester is enzymatic cleavage of the terminal ester bond with formation of a highly unstable hydroxymethyl ester which rapidly dissociates to the parent penicillin and formaldehyde (Scheme 1). A reason for the different enzymatic stabilities of the acyloxymethyl ester and simple alkyl esters of penicillins is certainly that the penicillin carboxyl group is highly sterically hindered. The terminal ester in the acyloxymethyl derivative is less hindered, and thus should be more accessible to enzymatic attack.



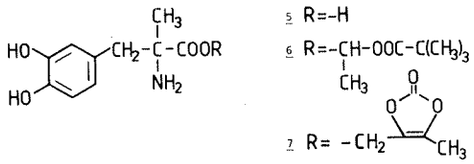
Scheme 1

The principle has been used successfully to improve the oral bioavailability of ampicillin (1), and no fewer than three ampicillin prodrug forms are now on the market, namely, the pivaloyloxymethyl ester (2) (pivampicillin) [27], the phthalidyl ester (3) (talampicillin) [28, 29] and the ethoxycarbonyloxyethyl ester (4) (bacampicillin) [30], the latter containing a terminal carbonate ester moiety. The properties of these prodrugs as well as of other similar acyloxyalkyl esters of β-lactam an-

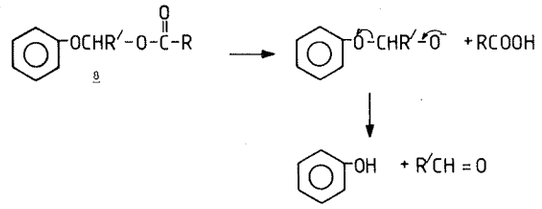


tibiotics, such as mecillinam and cephalosporins, have been reviewed extensively [24].

In more recent years the applicability of this double ester concept in prodrug design has been expanded further. Thus, similar esters have been prepared from indomethacin and other non-steroidal anti-inflammatory agents [31] as well as from cromoglycic acid [32], and found to be useful as prodrugs for enhancement of the dermal delivery of these acidic drugs. Other carboxylic acid agents where acyloxyalkyl esters have been developed as prodrugs include isoguvacine [33], methyldopa [34–36] and tyrosine [37]. Whereas methyldopa (5) is variably and incompletely absorbed its pivaloyloxyethyl ester (6) is almost completely and more uniformly absorbed in man following oral administration and is hydrolyzed rapidly on the first pass to the parent drug [35, 36]. A different ester type of methyldopa, a (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl derivative (7), was recently reported to be another potentially useful prodrug for improving the oral bioavailability [38]. A similar ester type of ampicillin has been described recently and shown to be an orally well absorbed prodrug [38a].



The applicability of acyloxyalkyl esters as biologically reversible transport forms has been extended to include phenolic drugs, the derivatives being acyloxyalkyl ethers (8). Bodor and co-workers [93, 94] have recently prepared such acyloxyalkyl ethers of various phenols (e.g., β-estradiol and phenylephrine), thiophenol and catechols (e.g., dopamine and epinephrine). The derivatives are hydrolyzed by a sequential reaction involving the formation of an unstable hemiacetal intermediate (Scheme 2) and they are as susceptible as normal phenol esters to undergo enzymatic hydrolysis by, e.g., human plasma enzymes. However, the acyloxyalkyl ethers ap-

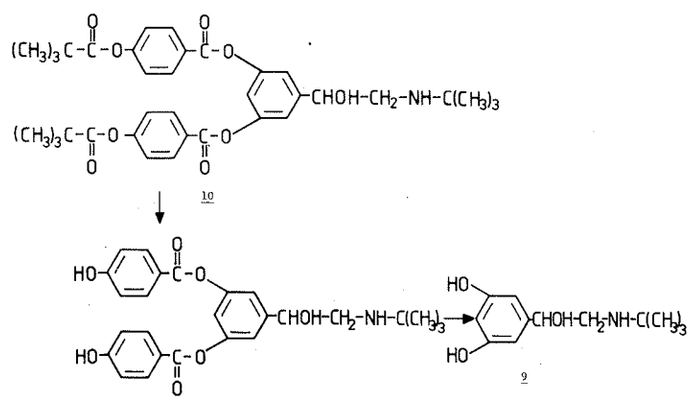


Scheme 2

pear to be more stable against chemical (hydroxide ion-catalyzed) hydrolysis than phenolate esters, and this may make them more favourable in prodrug design [93].

According to their cleavage mechanism, acyloxyalkyl esters and ethers can be considered as double prodrugs (pro-prodrugs). An interesting variant of the double ester prodrug concept is provided in the work by Olsson and co-workers [95, 96] on terbutaline (9). In order to achieve increased absorption, reduced first-pass metabolism and prolonged duration of action, a *p*-pivaloyloxybenzoate double ester prodrug (10) was made. Since the pivaloyl ester group is the most susceptible to undergo enzymatic hydrolysis, it was expected that the prodrug would undergo first-pass hydrolysis preferentially at the *p*-pivaloyloxy bond, followed by conjugation reactions with sulphuric and glucuronic acid at the resulting *p*-hydroxybenzoyl moiety (Scheme 3). In this way the active resorcinol moiety in terbutaline would be protected during first-pass and free terbutaline may be generated from hydrolysis of the conjugated or free *p*-hydroxybenzoate during and after the distribution phase. Experimental support for the cascade ester to function in this way was obtained and prolonged terbutaline plasma profiles were observed in dogs with this prodrug [96].

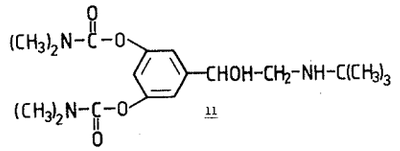
Carbamate esters may be promising prodrug candidates for phenolic drugs. The



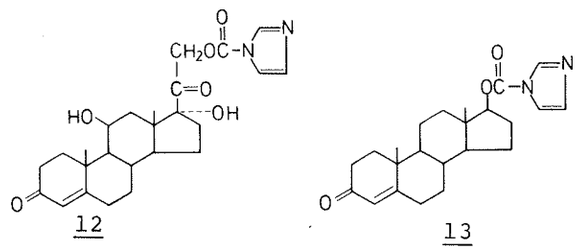
Scheme 3

bis-*N,N*-dimethylcarbamate of terbutaline (11) was also examined in the work by Olsson and Svensson [96] referred to above. It showed a half-life of hydrolysis in human plasma of about 10 hours, and this relatively high stability was partly due to the fact that the compound inhibited its own hydrolysis by reversible binding to plasma esterases. As a result of the improved hydrolytic stability, the prodrug survived the first-pass hydrolysis in the dog to a substantial degree and produced sustained blood levels of the parent drug in the dog following a single oral dose.

The enzymatic hydrolytic behaviour of carbamate esters has been examined by Digenis and Swintosky [6]. *N*-Unsubstituted or -monosubstituted carbamates derived from phenols showed high lability and strong enzymatic catalysis whereas most *N*-disubstituted carbamates proved highly stable, as did carbamates of aliphatic hydroxy compounds. The kinetics and mechanism of the non-enzymatic hydrolysis of carbamates have been studied thoroughly [97 – 102].



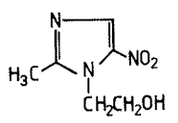
Whereas carbamates of alcohols in general appear to be of no value in prodrug design due to their high stability, certain activated carbamates may be useful. Imidazole-1-carboxylic acid esters belong to this category and such derivatives of hydrocortisone (12) and testosterone (13) have been shown recently to undergo a relatively facile hydrolysis in aqueous buffer solutions [103]. At pH 7.4 and 37°C the half-life of hydrolysis of the hydrocortisone derivative was found to be 8 minutes and that for the testosterone derivative 65 hours, the different reactivity being ascribed to the different steric hindrance in the alcohol portions of the steroids. No enzymatic catalysis by human plasma was observed. Due to protonation of the imidazole group ($pK_a \approx 3.5$) the derivatives showed increased solubility in acidic aqueous solution relative to the parent steroids [103].



Ester formation has long been recognized as an effective means of increasing the aqueous solubility of drugs containing a hydroxyl group, with the aim of developing prodrug preparations suitable for parenteral administraton. Two physicochemical

strategies can be employed to increase aqueous solubility: (i) introduction of an ionic or ionizable group by the pro-moiety and (ii) derivatization in such a manner that the prodrug shows a decreased melting point [104].

The most commonly used esters for increasing aqueous solubility of alcoholic drugs are hemisuccinates, phosphates, dialkylaminoacetates and amino acid esters. However, their use is not without problems, considering the ideal properties of such prodrugs: they should possess adequate aqueous solubility, sufficient aqueous solution stability to allow long-term storage of its solution (i.e., 2 years at room temperature) and yet they should be converted rapidly in vivo to the active parent drug. For example, succinate esters are not good substrates for hydrolytic enzymes [15] and often show relatively slow and incomplete cleavage in vivo, as has been described for such esters of various corticosteroids [105, 106] and chloramphenicol [82, 107 – 109]. Besides, their solution stability is limited due to intramolecular reactions (e.g., catalysis of ester hydrolysis or *O*-acyl migration in corticosteroids) of the terminal succinate carboxyl group [110, 111]. Phosphate esters as sodium salts are freely water-soluble and are so stable in vitro that solutions with practical shelf-lives often can be formulated [112 – 115]. Thus, a shelf-life of more than 10 years for an aqueous solution of vidarabine-5′-phosphate at pH 6.8 and 25°C has been predicted [113]. They are also rapidly hydrolyzed enzymatically in vivo (e.g., Refs. 116 – 118, 122), although exceptions exist. Thus, the phosphate ester (15) of metronidazole (14) shows a rather slow rate of conversion in human serum, the hydrolysis exhibiting apparent zero-order kinetics [79]. For the third type of water-soluble ester derivatives, i.e., esters with an ionizable amino function in the acid portion, only sparse information is available on their enzymatic hydrolysis. Bundgaard et al. [80, 81] have prepared eight amino acid esters of metronidazole (14) and evaluated their potentiality as water-soluble parenteral delivery forms of the parent drug whose solubility in water is limited (≈ 1% w/v). Hydrochloride salts of all the esters exhibited a water solubility greater than 20% w/v but their susceptibility to undergo enzymatic hydrolysis varied widely, as seen from the data in Table 3. Due to its facile cleavage in plasma, excellent solubility properties (> 50% w/v in water) and ease of synthesis and purification, the hydrochloride salt of metronidazole *N,N*-dimethylglycinate (16) appeared to be the most promising prodrug candidate [80]. Following intravenous administration to dogs the ester was converted rapidly ($t_{1/2}$



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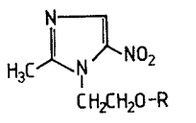


15 R = $-\overset{\overset{O}{\|}}{P}-(OH)_2$

16 R = $-\overset{\overset{O}{\|}}{C}-CH_2-N\begin{matrix}CH_3\\CH_3\end{matrix}$

≈ 5 min) and completely to metronidazole [81]. It is of interest to compare the in vivo half-life in dogs (5 minutes) to that observed in vitro in dog plasma (25 minutes). A disadvantage of this prodrug is that it is not sufficiently stable for formulation as a ready-to-use solution [81] and must be used as a formulation to be reconstituted as a solution prior to use. Recently, some kinds of water-soluble amino acid 21-esters of corticosteroids possessing both a high in vitro stability and a high susceptibility of undergoing enzymatic hydrolysis have been developed by Anderson et al. [119 – 121].

TABLE 3
Half-lives for the Hydrolysis of Various Amino Acid Esters of Metronidazole in 80% Human Plasma (pH 7.4) and 0.05 M Phosphate Buffer (pH 7.40) at 37°C [a]

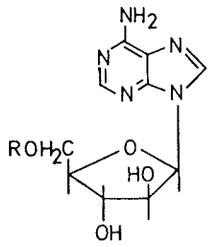
Ester	$t_{1/2}$ in human plasma (minutes)	$t_{1/2}$ in buffer (minutes)
N,N-Dimethylglycinate	12	250
Glycinate	41	115
N-Propylglycinate	8	90
3-Aminopropionate	207	315
3-Dimethylaminopropionate	46	52
3-Dimethylaminobutyrate	334	580
4-Morpholinoacetate	30	1880
4-Methyl-1-piperazinoacetate	523	1720

a. From Bundgaard et al. [80].

Sulphate esters of alcohols and phenols have long been considered as prodrug forms useful for obtaining injectable preparations [14]. However, recent studies indicate that such esters may be very resistant to undergoing hydrolysis in vivo and, accordingly, would not be suitable prodrugs. Thus, Miyabo et al. [122] found that dexamethasone-21-sulphate produced virtually no free dexamethasone in plasma and urine following intravenous injection in man, but was excreted largely unchanged in urine. Similarly, Williams et al. [123] have found that the sulphate esters of paracetamol and 3-hydroxymethyl-phenytoin do not generate the parent drugs when administered parenterally to mice or rats.

A high crystal lattice energy of solid compounds, as manifested in a high melting point, results in poor solubility (in all solvents). Therefore, an approach to reduce this energy may result in improved aqueous solubility. An example of the usefulness of this approach in prodrug design concerns vidarabine (17). It has a low water solubility (0.5 mg ml^{-1}), primarily due to the occurrence of intermolecular hydrogen bonding in the crystalline state, as reflected in its melting point of 260°C. By esterification of the 5′-hydroxyl group this bonding is reduced, and, further, by choosing an only slightly lipophilic acyl group such as formyl a vidarabine ester with

greatly increased aqueous solubility has been obtained [68]. The 5′-formate ester (18) is hydrolyzed rapidly in human blood with a half-life of about 6 – 8 minutes, and it appears to be a useful parenteral delivery form of vidarabine [68]. Several other examples of using this approach to increase the aqueous solubilities of drugs are given in other sections of this chapter.



17 R = H

18 R = -C(=O)-H

3. Prodrugs for amides, imides and other NH-acidic compounds

3.1. *N*-Mannich bases

N-Mannich bases have been proposed as potentially useful prodrug candidates for NH-acidic compounds such as various amides, imides, carbamates, hydantoins and urea derivatives as well as for aliphatic or aromatic amines [12, 124 – 132]. The compounds have been known for a long time and the chemistry of their formation has been the subject of two reviews [133, 134]. They are generally formed by reacting an NH-acidic compound with formaldehyde or, in very rare cases, other aldehydes and a primary or secondary aliphatic or aromatic amine. The process can be considered as an *N*-aminomethylation or *N*-amidomethylation (in the case of the NH-acidic component being an amide; Scheme 4).

$$R\text{-}CONH_2 + CH_2O + R_1R_2NH \rightleftharpoons R\text{-}CONH\text{-}CH_2\text{-}NR_1R_2 + H_2O$$

Scheme 4

3.1.1. Hydrolysis of N-Mannich bases

The kinetics of decomposition of a great number of *N*-Mannich bases in aqueous solution has been the subject of several studies [124 – 126, 128 – 132]. At constant pH and temperature, the decomposition rates of the *N*-Mannich bases followed strict first-order kinetics and all reactions went to completion. No general acid-base catalysis by the buffers used was apparent. The pH-rate profiles for most compounds have a sigmoidal shape, as seen in Figure 1. These pH dependences of the observed apparent first-order rate constant, k_{obs}, could be accounted for by assuming spontaneous decomposition of the free Mannich bases (B) and their conjugate acids (BH^+); the expression for k_{obs} is:

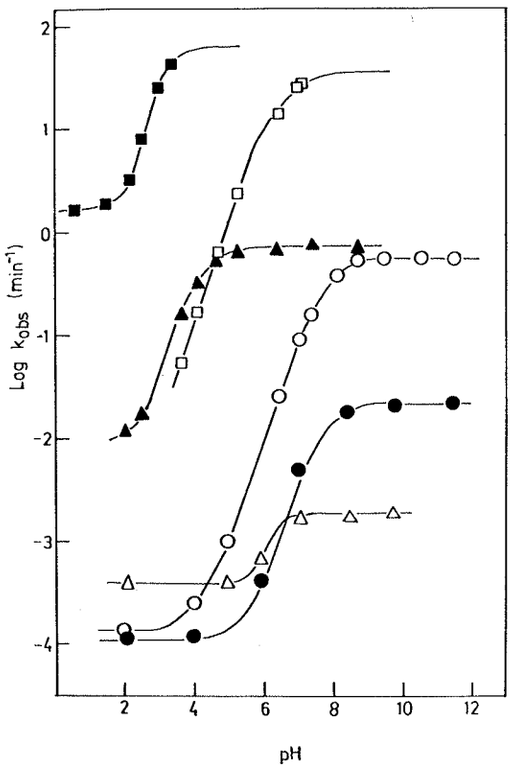


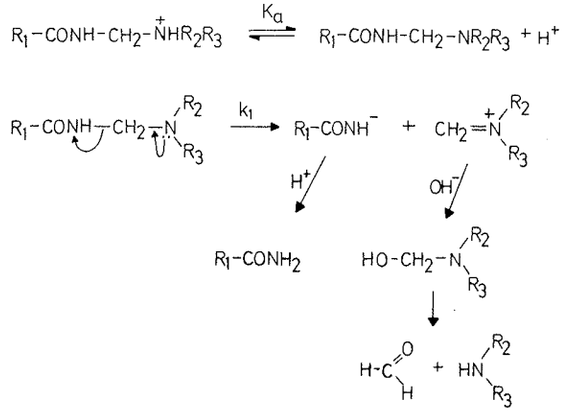
Fig. 1. The pH-rate profiles for the decomposition of various *N*-Mannich bases in aqueous solution at 37°C. ■, *N*-(Morpholinomethyl)-*p*-toluenesulphonamide; □, *N*-(piperidinomethyl)trichloroacetamide; ▲, *N*-(morpholinomethyl)trichloroacetamide; ○, *N*-(diethylaminomethyl)benzamide; ●, *N*-(isobutylaminomethyl)benzamide; △, *N*-(benzylaminomethyl)benzamide. From Bundgaard and Johansen [125].

$$k_{obs} = \frac{k_1 K_a}{a_H + K_a} + \frac{k_2 a_H}{a_H + K_a} \quad (1)$$

where K_a is the apparent ionization constant of the protonated *N*-Mannich bases, a_H is the hydrogen ion activity, and k_1 and k_2 are the apparent first-order rate constants for the spontaneous degradation of B and BH^+, respectively.

The reaction mechanism proposed [124, 125] for the decomposition involves as rate-determining step an unimolecular N-C bond cleavage with formation of an amide (or imide) anion and an immonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the anion and a hydroxide ion to the immonium ion,

giving methylolamine, which rapidly dissociates to formaldehyde and amine (Scheme 5). Loudon et al. [135] have independently proposed a similar mechanism for *N*-Mannich bases derived from isopropylaldehyde.



Scheme 5

3.1.1.1. Structural effects on decomposition rate. The structural effects on the decomposition rate of *N*-Mannich bases derived from carboxamides, thioamides, sulphonamides or imides and aliphatic or aromatic amines involve steric effects and basicity of the amine component and acidity of the amide-type component [125, 129, 132]. These factors are most pronounced with respect to the rate constant, k_1, and, accordingly, to the decomposition rate in weakly acidic to basic aqueous solutions. The rates of the hydrolysis of unprotonated Mannich bases are accelerated strongly by (a) increasing steric effects within the amine substituent, (b) increasing basicity of the amine component and (c) increasing acidity of the parent amide-type compound.

For some *N*-Mannich bases of benzamide and various amines the rate constant k_1 can be expressed by the following equation [125]:

$$\log k_1 = 2.30\nu - 3.50 \qquad (k_1 \text{ in } \text{min}^{-1};\ 37°\text{C}) \tag{2}$$

where ν is Charton's steric substituent parameter for alkyl amino groups. The marked influence of the steric effect on k_1 can be exemplified by comparing the k_1 values for the benzamide Mannich bases of diethylamine (0.52 min^{-1}) and ethylamine (0.0084 min^{-1}).

For amines with the same steric properties but differing in basicity, the rate constants k_1 for the decomposition of the respective *N*-Mannich bases were shown to increase almost 10-fold with an increase of unity of the pK_a of the amines. Thus, for various *N*-(arylaminomethyl)-succinimide derivatives (19) the following relationship was derived [129]:

$$\log k_1 = 0.93\ pK_a - 4.81 \qquad (k_1 \text{ in } \text{min}^{-1};\ 37°\text{C}) \tag{3}$$

The structural effect of the amide-type component in the Mannich bases on the decomposition rate was delineated from rate data obtained for several Mannich bases with either piperidine or morpholine [125]. The reactivity was shown to increase strongly with increasing acidity of the parent amide-type compound. For the Mannich bases with piperidine the following relationship was derived:

$$\log k_1 = -1.42\ pK_a + 19.3 \qquad (k_1 \text{ in } \text{min}^{-1};\ 37°\text{C}) \tag{4}$$

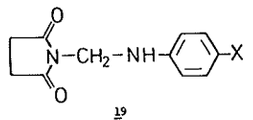
For morpholine derivatives, Eqn. 5 was obtained:

$$\log k_1 = -1.15\ pK_a + 13.9 \qquad (k_1 \text{ in } \text{min}^{-1};\ 37°\text{C}) \tag{5}$$

Eqns. 4 and 5, in which pK_a refers to the ionization constant for the parent amide-type compounds (at 20 – 25°C), cover both aromatic and aliphatic carboxamides as well as a thioamide and a sulphonamide. *N*-Mannich bases of urea, thiourea and *N*-acyl thiourea derivatives were found to deviate from these relationships, showing a greater reactivity than expected on the basis of their pK_a values. The *N*-Mannich base (20) formed between (–)-ephedrine and benzamide is also more reactive than predicted, the half-life of hydrolysis at pH 7.4 and 37°C being 2.2 minutes [132]. A positive deviation was further observed with *N*-Mannich bases of salicylamide [128]. Despite these exceptions the structure-reactivity relationships obtained have been used successfully to predict the reactivity of new *N*-Mannich bases [138, 140].

Some representative rate data for the decomposition of various *N*-Mannich bases are given in Table 4.

The breakdown of the *N*-Mannich bases does not rely on enzymatic catalysis and identical decomposition rates were observed in solutions with or without addition of human plasma [137, 138].



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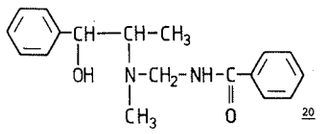

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TABLE 4
Rate Data for the Decomposition of Various *N*-Mannich Bases in Aqueous Solution at 37°C [a]

Compound	k_1 (min^{-1})	$t_{½}$ at pH 7.40 (minutes)
N-(Piperidinomethyl)benzamide	0.051	47
N-(Piperidinomethyl)-4-nitrobenzamide	0.17	8.0
N-(Piperidinomethyl)acetamide	0.0055	400
N-(Piperidinomethyl)dichloroacetamide	2.48	0.4
N-(Piperidinomethyl)trichloroacetamide	35	0.02
N-(Piperidinomethyl)nicotinamide	0.17	8.0
N-(Piperidinomethyl)thiobenzamide	13	0.06
N-(Morpholinomethyl)benzamide	0.0005	1400
N-(Morpholinomethyl)thiobenzamide	0.52	1.3
N-(Morpholinomethyl)-*p*-toluenesulphonamide	60	0.01
N-(Phenethylaminomethyl)benzamide[b]	0.0048	205
N-(Phenylpropanolaminomethyl)benzamide[b]	0.0031	225
N-(Methylaminomethyl)benzamide	0.0026	600
N-(Ethylaminomethyl)benzamide	0.0084	190
N-(Diethylaminomethyl)benzamide	0.52	4.0
N-(Dimethylaminomethyl)benzamide	0.032	58
N-(Benzylaminomethyl)benzamide	0.0020	380
N-(Morpholinomethyl)-*N'*-acetylthiourea	0.91	0.8
N-(Piperidinomethyl)-*N'*-methylurea	–	5.0
N-(Methylaminomethyl)salicylamide[c]	–	28
N-(Piperidinomethyl)salicylamide[c]	–	14
N-(Morpholinomethyl)salicylamide[c]	–	41
N-(α-Alaninomethyl)salicylamide[c]	–	17
N-(Anilinomethyl)succinimide[d]	0.36	1.9
N-(*p*-Toluidinomethyl)succinimide[d]	0.76	0.9

a. From Bundgaard and Johansen [125], unless otherwise indicated.
b. From Johansen and Bundgaard [132].
c. From Johansen and Bundgaard [128].
d. From Bundgaard and Johansen [129].

N-Mannich bases derived from aldehydes other than formaldehyde are not readily prepared, and therefore knowledge of the reactivity of such compounds is limited. Loudon et al. [135] have studied the hydrolysis of *N*-Mannich bases derived from some amides and isopropylaldehyde, and from the data reported it can be estimated that isopropylaldehyde Mannich bases are approximately 10 times more reactive in neutral and alkaline solutions than the corresponding *N*-Mannich bases with formaldehyde. Similarly, *N*-Mannich bases derived from amides and benzaldehyde are markedly more reactive than those prepared with formaldehyde [136].

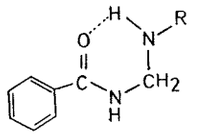
3.1.2. Application of N-Mannich bases as potential prodrugs

By appropriate selection of the amine component, it should be feasible to obtain

prodrugs of a given amide-type drug with varying degrees of in vivo lability. Besides, other physicochemical properties such as aqueous solubility, dissolution rate and lipophilicity can be modified for the parent compounds [12, 127].

Transformation of an amide into an *N*-Mannich base introduces a readily ionizable amino moiety which may allow the preparation of derivatives with greatly increased water solubilities at slightly acidic pH values where, as a matter of fortune, the stability may be quite high. This has been shown for various *N*-Mannich bases using benzamide as a model compound [127]. Whereas *N*-Mannich bases prepared from secondary amines showed very high solubilities in salt form, the Mannich bases derived from primary amines did not show increased solubility even as salts. This different behaviour was attributed to the occurrence of intramolecular hydrogen bonding in the latter derivatives (21).

The concept of *N*-Mannich base formation of NH-acidic compounds to yield more soluble prodrugs has already been utilized in the case of rolitetracycline (22). This highly water-soluble *N*-Mannich base of tetracycline and pyrrolidine which is used clinically is decomposed quantitatively to tetracycline in neutral aqueous solution, the half-life being 40 minutes at pH 7.4 and 37°C [139]. Similarly, various *N*-Mannich bases of carbamazepine (23) have been developed as water-soluble prodrugs for parenteral administration [140] (Table 5). The solubility of the hydrochloride salts of these *N*-Mannich bases in water was found to exceed 50%



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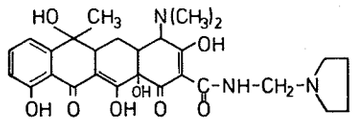

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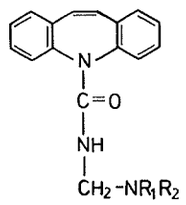

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w/v, i.e., more than 10^4-fold greater solubility than the parent drug. Following intramuscular administration in rats, higher and more rapidly appearing carbamazepine plasma levels were observed from aqueous solutions of the dipropylamino *N*-Mannich base prodrug than from administering a suspension of the parent drug [140].

TABLE 5
Ionization Constants and Rate Data for the Decomposition of Various *N*-Mannich Bases of Carbamazepine in Aqueous Solution at 37°C [a]

Compound	pK_a	k_1 (min^{-1})	k_2 (min^{-1})	$t_{½}$ at pH 7.40 (minutes)
N-(Diethylaminomethyl)carbamazepine	7.95	0.17	0.0017	19
N-(Dipropylaminomethyl)carbamazepine	7.75	0.27	0.0030	7
N-(Piperidinomethyl)carbamazepine	7.90	0.015	0.0011	165

a. From Bundgaard et al. [140].

In addition, the concept may be useful for improving the dissolution behaviour of poorly soluble drugs in an effort to improve the oral bioavailability. Thus, *N*-Mannich bases of various NH-acidic compounds (e.g., phthalimide, chlorzoxazone, phenytoin, barbital, *p*-toluenesulphonamide, acetazolamide, chlorothiazide and allopurinol) with morpholine or piperidine as the amine component were found to possess markedly greater (up to a factor of 2,000) intrinsic dissolution rates in 0.1 M hydrochloric acid in comparison with the parent compounds [126, 131]. Once dissolved, the *N*-Mannich bases are cleaved very rapidly, with the quantitative release of the parent compounds.

3.2. *N*-HYDROXYMETHYL DERIVATIVES

When an NH-acidic compound is allowed to react with formaldehyde in absence of a primary or secondary amine *N*-hydroxymethylation occurs (Scheme 6):

$$R\text{-}CONH_2 + CH_2O \rightleftharpoons R\text{-}CONH\text{-}CH_2OH$$

Scheme 6

The kinetics of decomposition of a large number of *N*-hydroxymethylated amides, imides, carbamates and hydantoins in aqueous solution has been studied [141, 142]. It was found that the decomposition exhibited a first-order dependence on hydroxide ion concentration up to a pH about 12 and that the rates increased sharply with increasing acidity of the parent compound (Fig. 2). The following

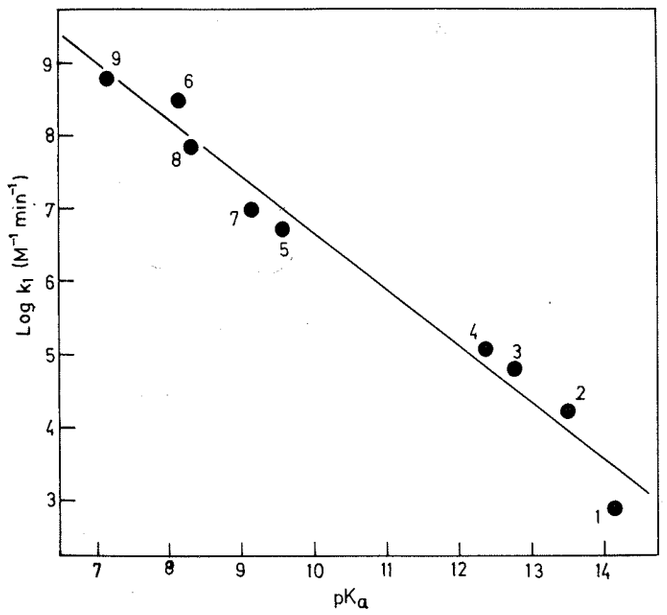


Fig. 2. Plot of the logarithm of the apparent hydroxide ion catalytic rate constants (k_1) for decomposition of various *N*-hydroxymethyl derivatives at 37°C against pK_a of the parent compounds. 1, Chloroacetamide; 2, dichloroacetamide; 3, thiobenzamide; 4, trichloroacetamide; 5, succinimide; 6, 5-chloro-2-benzoxazolinone (chlorzoxazone); 7, 5,5-dimethylhydantoin; 8, phenytoin; 9, nitrofurantoin. From Bundgaard and Johansen [142].

linear correlation was found between log k_1 (where k_1 is the apparent hydroxide ion catalytic rate constant) and pK_a for the above-mentioned group of compounds:

$$\log k_1 = -0.77\ pK_a + 14.4 \qquad (k_1 \text{ in } M^{-1}\ min^{-1};\ 37°C) \tag{6}$$

Eqn. 6 may be expressed in a more directly useful form:

$$\log t_{½} = 0.77\ pK_a - 8.34 \qquad (r = 0.986;\ n = 9) \tag{7}$$

where $t_{½}$ is the half-life for hydrolysis at pH 7.4 and 37°C (in minutes) and pK_a refers to the ionization constant of the parent NH-acidic compound.

This relationship allows one to predict the reactivity of an *N*-hydroxymethyl derivative solely from a knowledge of the pK_a of the parent compound. Thus, it can be predicted that the requirement for a half-life of the decomposition reaction of less than 1 hour at pH 7.4 and 37°C is that the parent NH-acidic compound possesses a pK_a value of less than 13.1 or that a pK_a value of less than 10.8 is required for a half-life of less than 1 minute.

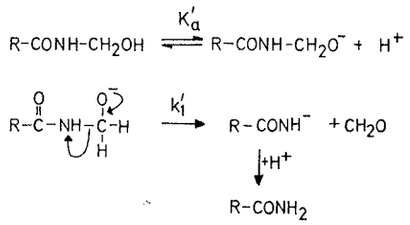
TABLE 6
Apparent Hydroxide Ion Catalytic Rate Constants (k_1) for the Decomposition of Various *N*-Hydroxymethyl Derivatives in Aqueous Solution at 37°C, Half-lives of Decomposition at pH 7.40 and 37°C and pK_a Values of the Parent NH-Acidic Compounds[a]

N-Hydroxymethyl derivative of:	k_1 (M^{-1} min^{-1})	$t_{½}$	pK_a
Benzamide	1.1×10^2	183 h	14 – 15
4-Chlorobenzamide	2.2×10^2	88 h	
4-Nitrobenzamide	8.9×10^2	22 h	
3-Nitrobenzamide	7.4×10^2	27 h	
4-Methoxybenzamide	6.0×10	120 h	
3-Methoxybenzamide	1.6×10^2	317 h	
Nicotinamide	5.1×10^2	37 h	
Thiobenzamide	6.3×10^4	18 min	12.8
Chloroacetamide	6.9×10^2	28 h	14.2
Dichloroacetamide	1.5×10^4	77 min	13.5
Trichloroacetamide	1.2×10^5	10 min	12.4
Succinimide	5.0×10^6	14 sec	9.6
Phthalimide	2.5×10^6	28 sec	8.3
5-Chloro-2-benzoxazolinone	3.5×10^8	0.2 sec	8.2
Nitrofurantoin	6.2×10^8	0.1 sec	7.2
Phenytoin	7.1×10^7	1.0 sec	8.3
5,5-Dimethylhydantoin	1.0×10^7	6.9 sec	9.2

a. From Bundgaard and Johansen [141, 142].

A selection of half-lives for decomposition of various *N*-hydroxymethyl derivatives at pH 7.4 and 37°C are given in Table 6. Most values were calculated using rate constants determined at higher pH values.

It has been established that the reaction mechanism for the decomposition of the *N*-hydroxymethyl derivatives of amides and imides involves a stepwise pathway, with an *N*-hydroxymethyl anion as an intermediate undergoing rate-determining N-C bond cleavage, as illustrated in Scheme 7 [141]. Bansal et al. [143] have shown that similar rate-determining transition states are involved in the formation of *N*-hydroxymethyl derivatives of uracils. As can be expected on the basis of this reaction mechanism, the rate of decomposition of *N*-hydroxymethyl derivatives is not catalyzed by enzymes [137].



Scheme 7

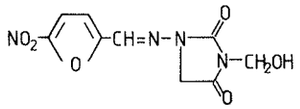
The *N*-hydroxymethyl derivatives of amide- or imide-type compounds are more water-soluble than the parent compounds [141 – 144], thus suggesting a potential area of application of *N*-hydroxymethyl prodrugs for nitrogenous molecules, e.g., for increasing dissolution rates, and hence oral bioavailability. By replacing a proton bound to a nitrogen atom by a hydroxymethyl group intra- or intermolecular hydrogen bonding in such molecules may be decreased, leading to a corresponding decrease in melting point and increase in water-solubility [142 – 144]. This is illustrated by the data given in Table 7.

TABLE 7
Melting Points and Aqueous Solubilities (at 22 – 25°C) of Various NH-Acidic Compounds and Their *N*-Hydroxymethyl Derivatives

Compound	Melting point (°C)	Aqueous solubility (M)	Reference
Benzamide	130	0.11	141
N-Hydroxymethylbenzamide	104 – 106	0.30	141
Thiobenzamide		0.012	141
N-Hydroxymethylthiobenzamide	64 – 65	0.073	141
Chlorzoxazone	191	0.0014[a]	141
N-Hydroxymethylchlorzoxazone	138 – 144	0.0020[a]	141
Phenytoin	295 – 298	0.00013[a]	142
3-Hydroxymethylphenytoin	198 – 199	0.0037[a]	142
Nitrofurantoin	270 – 272	0.00046[a]	142
3-Hydroxymethylnitrofurantoin	–	0.0012[a]	142
Uracil	340	0.03	143
1,3-Dihydroxymethyluracil	101	2.90	143
Glutethimide	85	0.005	144
N-Hydroxymethylglutethimide	82	0.022	144

a. In 0.1 M hydrochloric acid.

3-Hydroxymethylnitrofurantoin (24) is used as a faster dissolving and absorbing prodrug of nitrofurantoin [145]. Several *N*-hydroxymethyl derivatives, such as 3-hydroxymethyl-5,5-dimethylhydantoin [146, 147], [bis-*N*,*N*′-(5-ureido-2,4-diketotetrahydroimidazole)-*N*,*N*′-dimethylol] (Germall 115®) and *N*-methyl-*N*′-hydroxymethylthiourea (noxythiolin), are used widely as preservatives in medical and cosmetic formulations. The compounds are odourless solids constituting a source of antibacterially active formaldehyde which is released gradually from the derivatives when these go into solution [142]. The kinetics of hydrolysis of Germall 115 [148] and noxythiolin [149, 150] has been studied recently.



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From the structure-reactivity data given above it is readily evident that *N*-hydroxymethylation is not a universally applicable approach to bioreversible derivatization of NH-acidic compounds but is limited to compounds possessing a pK_a value of less than about 10.5 – 11 in order to provide a sufficient rate of drug regeneration at physiological pH. For example, *N*-hydroxymethyl derivatives of carboxamides (pK_a 14 – 15) are relatively stable, the half-lives for decomposition of the derivates of, e.g., benzamide and nicotinamide being 183 and 37 hours, respectively, at pH 7.4 and 37°C [141]. Derivatives obtained from aldehydes other than formaldehyde possess, however, a greater lability [136] but their availability is seriously restricted. Whereas *N*-hydroxymethyl derivatives are prepared easily by reacting formaldehyde with the NH-acidic compound in water, ethanol or other solvents most aliphatic and all aromatic aldehydes do not behave as formaldehyde does [151]. The reaction with, e.g., amides usually does not stop at the *N*-alkylol stage, RCONHCHOHR′, but progresses further to yield the stable alkylidene- or arylidene-bisamide, $(RCONH)_2CHR'$. The only exceptions are the α-halogenated aldehydes such as chloral [151 – 153] and also acetaldehyde toward thiobenzamide [154]. A recent study [136] on the kinetics of hydrolysis of such hydroxyalkyl compounds derived from benzamide, thiobenzamide and chloral or acetaldehyde as well as of *N*-(α-hydroxybenzyl)benzamide (25), the latter being obtained from hydrolysis of the *N*-Mannich base *N*-(α-morpholinobenzyl)benzamide [136], has shown that these derivatives are much more unstable in neutral and alkaline solutions than are the corresponding *N*-hydroxymethyl derivatives (Table 8). Thus, whereas the half-life of decomposition of *N*-(hydroxymethyl)benzamide is 183 hours at pH 7.4 and 37°C the half-life for the benzamide compound derived from benzaldehyde (25) is

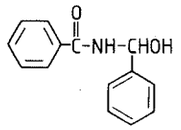

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TABLE 8

Apparent Hydroxide Ion Catalytic Rate Constants (k_{OH}) for the Decomposition of Various *N*-Hydroxyalkyl Derivatives in Aqueous Solution (μ = 0.5; 37°C), and Half-lives ($t_{1/2}$) of Decomposition at pH 7.40 and 37°C [a]

Compound	k_{OH} (M^{-1} min^{-1})	$t_{1/2}$ (minutes)
N-(α-Hydroxybenzyl)benzamide	1.8×10^5	6.5
N-(α-Hydroxy-2,2,2-trichloroethyl)benzamide	1.7×10^3	6.9×10^2
N-(Hydroxymethyl)benzamide	1.1×10^2	1.1×10^4
N-(α-Hydroxyethyl)thiobenzamide	2.3×10^6	0.5
N-(α-Hydroxy-2,2,2-trichloroethyl)thiobenzamide	4.5×10^6	0.3
N-(Hydroxymethyl)thiobenzamide	6.3×10^4	18

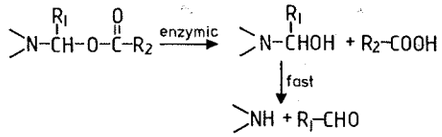
a. From Bundgaard and Johansen [136].

only 6.5 minutes under the same conditions. The difference in reactivity was suggested to be due primarily to steric effects within the α-substituents [136]. The implications of these results for the design of *N*-acyloxyalkyl-type prodrugs are discussed below (section 3.3).

Finally, it should be pointed out that although *N*-hydroxymethyl derivatives may be useful as such as prodrug forms the most important aspect of *N*-hydroxymethylation resides in the fact that the hydroxyl group introduced by this process is readily amenable to bioreversible derivatization, e.g., by esterification to produce water-soluble or lipophilic *N*-acyloxymethyl derivatives.

3.3. *N*-Acyloxyalkyl Derivatives

In recent years *N*-acyloxyalkylation has become a commonly used approach to obtain prodrugs of various amides, imides, hydantoins, uracils, tertiary or *N*-heterocyclic amines and other NH-acidic compounds [9 – 12, 155]. The usefulness of this approach stems from the facts that by varying the acyl portion of the derivatives it is possible to control the rate of regeneration of the parent drug and to obtain prodrugs with varying physicochemical properties, such as water-solubility or lipophilicity. Whereas the derivatives show good stability in aqueous solution in vitro similar to other esters, in general they are cleaved rapidly in vivo by virtue of enzyme-mediated hydrolysis. The regeneration of the parent NH-acidic drug takes place via a two-step reaction (Scheme 8). Enzymatic cleavage of the ester grouping results in the formation of an *N*-hydroxyalkyl derivative, which subsequently is assumed to decompose instantaneously into the corresponding aldehyde and the NH-acidic drug, as described in section 3.2. Thus, the rate of drug formation is dependent solely on the rate of the initial ester cleavage, which can be controlled by steric and electronic factors (see, e.g., Table 9).



Scheme 8

The most commonly used acyloxyalkyl derivatives are acyloxymethyl compounds, i.e., derivatives from which formaldehyde is released from an *N*-hydroxymethyl intermediate. As discussed above, the *N*-acyloxymethylation approach is limited to amide-type compounds possessing a pK_a value of less than about 10.5 – 11 when the requirement is to be fulfilled that the intermediate *N*-hydroxymethyl derivative should only have a transistory existence in the overall process of drug release, as outlined in Scheme 8. However, by using aldehydes other than formaldehyde, the

TABLE 9
Half-times ($t_{½}$) of the Conversion of Various *N*-Acyloxymethyl Derivatives of Allopurinol to the Parent Compound at 37°C, Water-solubilities (*S*) and Partition Coefficients (*P*)[a]

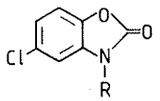
Compound	$t_{½}$		*S* (mg/ml)	log *P*[b]
	pH 7.4 buffer	80% human plasma (minutes)		
Allopurinol			0.50	−0.55
1-(Acetoxymethyl)allopurinol	87 h	31	0.58	−0.35
1-(Butyryloxymethyl)allopurinol	193 h	9	0.35	0.60
1-(Benzoyloxymethyl)allopurinol	237 h	4	0.024	1.50
1-(Nicotinoyloxymethyl)allopurinol	26 h	21	0.093	0.27
1,5-bis(Butyryloxymethyl)allopurinol	25 h	22	0.050	1.82
2,5-bis(Butyryloxymethyl)allopurinol	35 h	32	0.094	1.60
1-(*N,N*-Dimethylglycyloxymethyl)allopurinol, HCl	72 min	7	> 500	−0.49[c]
1-(*N,N*-Diethylglycyloxymethyl)allopurinol, HCl	49 min	10	> 500	0.20[c]
1-(*N,N*-Dipropylglycyloxymethyl)allopurinol, HCl	50 min	12	> 400	1.27[c]
1-(DL-Phenylalanyloxymethyl)allopurinol, HBr	40 min	9	> 200	0.40[c]
1-(L-Leucyloxymethyl)allopurinol, HBr	17 min	6	> 400	0.19[c]

a. From Bundgaard and Falch [171, 172].
b. Between octanol and water.
c. Between octanol and a borate buffer, pH 8.0.

N-α-alkylol intermediate thus formed would be more unstable than the *N*-methylol analogue, as also discussed above, hence expanding the usefulness of *N*-acyloxyalkylation as a means of obtaining prodrug forms of also weakly NH-acidic drugs such as amides, carbamates and urea derivatives ($pK_a > 14$) [136]. It should be pointed out that although the availability of *N*-hydroxyalkyl derivatives other than those derived from formaldehyde is very limited this does not restrict a broad utility of *N*-acyloxyalkyl derivatives as prodrug forms. The reason is that besides being obtainable by esterification of the intermediate *N*-hydroxyalkyl derivative such derivatives are readily – and most often – obtained by reacting the NH-acidic drug substance with an α-acyloxyalkyl halide (e.g., Refs. 155–159). The latter compounds are easily available from the reaction of acid halides with a variety of aldehydes, including, e.g., acetaldehyde and benzaldehyde besides formaldehyde [158–162].

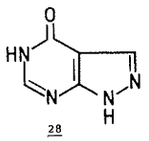
Several papers describing *N*-acyloxyalkylation as a means of obtaining prodrugs of various NH-acidic drugs have appeared in the last few years. By using this prodrug approach it has thus been possible to enhance the oral absorption of phenytoin [163] and the dermal delivery of theophylline [164], 6-mercaptopurine [165] and 5-fluorouracil [166]. The increased dermal absorption observed with the prodrug

derivatives is a result of the increased lipophilicity of the prodrugs along with the susceptibility of the derivatives to undergo enzymatic cleavage in the skin. In the case of 6-mercaptopurine some bioreversible *S*-acylaminomethyl and *S*-acyloxymethyl have also been prepared [165]. The extent to which water-solubility can be enhanced by *N*-acyloxyalkylation can be illustrated with chlorzoxazone (26). Its solubility in water is 0.2 mg ml^{-1}, while that of *N*-(*N'*,*N'*-dimethylglycyloxymethyl)chlorzoxazone hydrochloride (27) is about 200 mg ml^{-1} [167]. Similarly, various amino group-containing *N*-acyloxymethyl derivatives of phenytoin have been prepared and found to possess greatly increased aqueous solubility relative to the parent drug [115, 168]. Such compounds as well as the water-soluble disodium N_3-phosphoryloxymethyl-phenytoin have been shown recently to be potentially useful prodrugs of phenytoin, possessing improved oral and parenteral delivery characteristics in comparison to the parent drug [118, 168, 169].



26 R = -H

27 R = $-CH_2O-\overset{\overset{O}{\|}}{C}-CH_2N(CH_3)_2 \cdot HCl$



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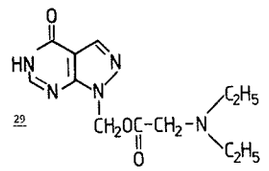
Besides being influenced by the hydrophilic or hydrophobic properties of the acyl moiety, the water-solubility and lipophilicity of *N*-acyloxyalkyl derivatives can be affected by the fact that the *N*-acyloxyalkylation may lead to decreased intermolecular hydrogen bonding in the crystal lattice. This is thus the case for the derivatives of theophylline [164], 5-fluorouracil [170] and allopurinol [171, 172]. A more detailed illustration of this phenomenon should be given for allopurinol (28). This compound is poorly soluble in water (0.5 mg ml^{-1}) and in various polar or apolar organic solvents. The X-ray interferogram of allopurinol shows a hydrogen bridge between the 1-NH group and 7-N of another molecule, while 2-N is bound to the hydrogen of a 5-NH group [173]. The strong crystal lattice energy of allopurinol due to these intermolecular hydrogen bonds, which is reflected in the high melting point of the compound (≈ 365°C), is certainly responsible for the poor solubility behaviour. By blocking the 1-NH, 2-NH or 5-NH groups by *N*-acyloxymethylation the intermolecular hydrogen bonding is decreased, as reflected in decreased melting points, and the water-solubility is increased. From the results given in Table 9 it can be seen that it is even feasible to obtain *N*-acyloxymethyl derivatives which at the same time are both more lipophilic than allopurinol and possess a higher water-solubility. The data in Table 9 also demonstrate the great

flexibility of the *N*-acyloxymethylation approach in that it is possible to modify greatly the cleavage rate, aqueous solubility and lipophilicity, and hence the delivery characteristics, by the appropriate selection of the acyl moiety of the prodrugs. Thus, selected derivatives, e.g., the derivative 29, have been shown to be useful for enhancing the rectal bioavailability of allopurinol or for obtaining an allopurinol preparation suitable for parenteral administration [172, 174, 174a]. The aqueous solubility of compound 29 as hydrochloride salt was found to be greater than 50% w/v [172].



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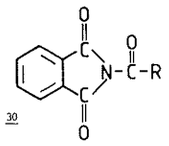
It should finally be mentioned that the rates of enzymatic hydrolysis of *N*-acyloxyalkyl derivatives of various NH-acidic compounds may depend greatly on the structure of the nitrogenous compound. Thus, whereas the half-life of hydrolysis of 7-pivaloyloxymethyl-theophylline in 10% human plasma at 37°C is only 3 minutes that of 1-pivaloyloxymethyl-5-fluorouracil is 38.5 hours under the same conditions [175].

3.4. *N*-Acyl derivatives

N-Acylation of amide- or imide-type compounds may be a useful prodrug approach in some particular cases, although this approach has received only little attention in the past.

Stella and Higuchi [176] studied the alkaline hydrolysis of various *N*-acyl phthalimides (30) and showed that regeneration of the parent phthalimide occurred only when the *N*-acyl carbonyl is sufficiently activated by electron-withdrawing substituents. Otherwise, the predominant reaction was ring-opening by imide hydrolysis.



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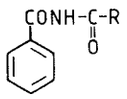

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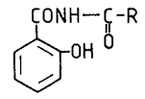

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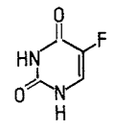
In assessing *N*-acylated amides or imides as potential prodrugs it is important to consider the possibility of enzymatic hydrolysis. Thus, it has been found that the rate of hydrolysis of *N*-acyl derivatives of benzamide (31) and salicylamide (32) is accelerated markedly by human plasma (Bundgaard, unpublished data).



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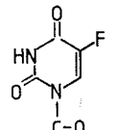

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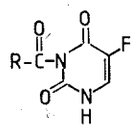

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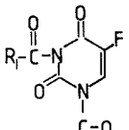

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Recently, studies have been carried out by Buur and Bundgaard [177 – 179] with the aim of improving the oral and rectal absorption characteristics of 5-fluorouracil (33) through development of bioreversible *N*-acyl derivatives with enhanced physicochemical properties in terms of delivery from the site of administration to the site of action within the body. Various N_1-acyl (34), N_3-acyl (35) and N_1,N_3-diacyl derivatives (36) were shown to be hydrolyzed readily in aqueous buffer solutions to yield 5-fluorouracil in quantitative amounts [177]. Whereas the N_1-acyl derivatives proved very unstable N_3-acylation was suggested to be a promising means of obtaining prodrug forms of 5-fluorouracil. As seen from the data in Table 10, the rate of N_3-deacylation is accelerated markedly in human plasma. In addition to being hydrolyzed rapidly under conditions similar to those prevailing in vivo, the hydrolysis rate being controllable by the appropriate selection of the acyl group, the N_3-acyl derivatives showed improved physicochemical properties of relevance to bioavailability as compared with 5-fluorouracil. As appears from Table 10, the derivatives are more lipophilic than 5-fluorouracil and, for some com-

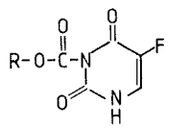
TABLE 10
Rate Data for the Hydrolysis of N_3-Acyl Derivatives of 5-Fluorouracil in Buffer (pH 7.4) and Human Plasma (37°C), as well as Aqueous Solubilities (*S*), Partition Coefficients (*P*) and Melting Points[a]

Compound	Melting point (°C)	S^b (mg/ml)	log P^c	$t_{½}$ (minutes)	
				Buffer	80% plasma
5-Fluorouracil (5-FU)	280 – 84	11.1	– 0.83		
3-Acetyl-5-FU	116 – 18	42.8	– 0.34	43	4.6
3-Propionyl-5-FU	113 – 14	35.3	0.19	50	20
3-Butyryl-5-FU	132 – 34	–	0.67	58	28
3-Benzoyl-5-FU	172 – 74	1.3	0.80	2900	110

a. From Buur and Bundgaard [177].
b. In acetate buffer, pH 4.0 (22°C).
c. Partition coefficients between octanol and acetate buffer, pH 4.0.

pounds, also more soluble in water. As referred to earlier for allopurinol, this apparently anomalous behaviour can be attributed to a decreased intermolecular hydrogen bonding in the crystal lattice, achieved by blocking the 3-NH group by the acylation and manifested in a pronounced melting point decrease [177].

In an additional study [178] various *N*-alkoxycarbonyl derivatives of 5-fluorouracil were examined. While the hydrolytic removal of an N_1-alkoxycarbonyl group proceeded very rapidly in aqueous buffer solutions, the N_3-alkoxycarbonyl group proved highly resistant towards chemical hydrolysis. However, the N_3-alkoxycarbonyl derivatives (37) showed enzyme-mediated cleavage in human plasma and, in particular, rat liver homogenate. Thus, N_3-phenyloxycarbonyl-5-fluorouracil was hydrolyzed to 5-fluorouracil with half-lives of 80 hours at pH 7.4 and 37°C, 390 minutes in 80% human plasma and 20 minutes in 30% rat liver homogenate [178]. It may be questioned whether the conversion of the N_3-alkoxycarbonyl derivatives to the parent drug is sufficiently facile in vivo.



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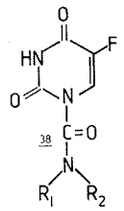

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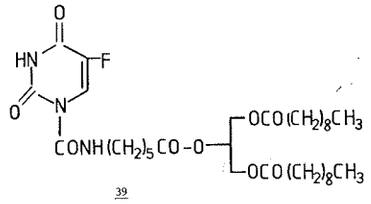
Another potential prodrug type of 5-fluorouracil belonging to the *N*-acyl category is carbamoyl derivatives. Various 1-carbamoyl-5-fluorouracil derivatives (38) (methyl-, ethyl-, butyryl-, phenyl- and *N,N*-dimethylcarbamoyl derivatives) [180, 181] were shown [179] to hydrolyze, to yield 5-fluorouracil in quantitative amounts, the half-lives of hydrolysis at pH 7.4 and 37°C being 8 – 11 minutes for the 1-alkylcarbamoyl derivatives and 5 seconds for the 1-phenylcarbamoyl derivative [179]. The *N,N*-dimethylcarbamoyl derivative proved to be highly stable. An interesting observation of concern in prodrug design was that the hydrolysis of the derivatives was inhibited markedly by human plasma. As serum albumin produced the same effect as plasma, this deceleration of the hydrolysis rate was attributed to a non-productive binding to or inclusion of the compounds by plasma proteins [179]. However, not all 1-carbamoyl derivatives of 5-fluorouracil behave in this way. Thus, the half-life of hydrolysis of the compound 39 in rat plasma in vitro was found to be 4 minutes and about 3 hours in buffer solution of pH 7.4 [182].

N-Acylation may also be a prodrug approach for various hydantoins, although no detailed information on the converting efficiency of the derivatives is available. 3-Acyl and 3-alkoxycarbonyl derivatives of nitrofurantoin have been described by English et al. [183] and, similarly, acyl and alkoxycarbonyl (at the 1- or 3-position) derivatives of phenytoin have been studied [184 – 187]. It appears that the N_1-acyl

derivatives are too resistant to function as prodrugs whereas the N_3-acyl derivatives are converted readily in vivo to the parent phenytoin.



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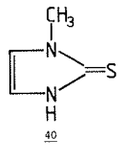
Methimazole (40) and carbimazole (41) are used widely as antithyroid drugs. Only relatively recently has it been shown that carbimazole is a prodrug of methimazole [188]. The ethoxycarbonyl group in carbimazole was found to be rapidly split off in vitro and in vivo by rat and human serum (conversion being complete after 3 minutes) and an early expectation [189] of carbimazole being a longer-acting drug is not valid. Jansson et al. [190] have shown that the oral bioavailability of the two compounds in humans is identical. Although there appears to be no therapeutic advantage by use of the prodrug (41), this example illustrates the chemical concept of *N*-acylation in prodrug design.



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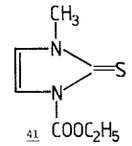

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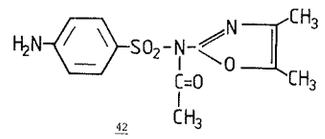
Finally, N_1-acylation of sulphonamides may be mentioned. The N_1-acetyl derivative (42) of sulphisoxazole is poorly soluble in water and is used as a taste-masking prodrug. It undergoes rapid hydrolysis in vivo to the parent sulphisoxazole [191]. A succinyl derivative of sulphamethoxydiazine has been prepared in order to obtain a highly water-soluble prodrug for parenteral use, but the derivative proved to be unsuitable due to too low stability in aqueous solution [192].



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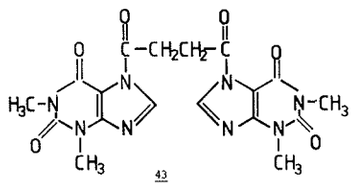
4. Prodrugs for amines

4.1. *N*-Acyl derivatives

N-Acylation of amines to give amide prodrugs has been used only to a limited extent due to the relative stability of amides in vivo. However, certain activated amides are

sufficiently chemically labile and, also, certain amides formed with amino acids may be susceptible to enzymatic cleavage in vivo.

7-Acylated derivatives of theophylline are activated amides (*N*-acyl imidazoles) and they hydrolyze very rapidly under non-enzymatic conditions [193, 194]. For example, 7-acetyltheophylline has a half-life in aqueous solution at 25°C of only 20 seconds. 7,7′-Succinylditheophylline (43) has been developed as an oral sustained-release prodrug of theophylline (44) [193]. It is much less soluble in water than the parent drug and possesses a slower intrinsic dissolution rate. The rate of hydrolysis of the prodrug to theophylline in solution is extremely fast ($t_{½} \approx 10$ seconds) and, thus, the rate-determining step in the release of theophylline from an oral solid dosage form containing the prodrug will be its dissolution rate [193 – 195]. The dissolution rate is pH-independent in the range of pH 1 – 8 and can be adjusted by the particle size of the compound. An advantage of this chemical controlled-release system over conventional controlled-release formulations may be that no accidental fast release of drug can occur as the slow dissolution rate is an intrinsic property of the prodrug.



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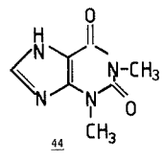

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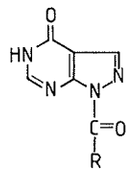


45 – 50

Acylation of the pyrazole moiety in allopurinol (28) similarly affords activated amide derivatives which may be useful as prodrugs. A series of N_1-acyl derivatives (45 – 50) of allopurinol have been synthesized recently and evaluated as potential prodrugs with the purpose of developing preparations suitable for rectal or parenteral administration [196]. Besides being easily cleaved to allopurinol in aqueous solutions at physiological pH, the derivatives are susceptible to a marked enzyme-catalyzed hydrolysis by human plasma (Table 11). The *N*-acyl derivatives were found to be more lipophilic than allopurinol, but the solubility in water was even greater (for the *N*-acetyl derivative) or only slightly reduced as compared with allopurinol (Table 11). This behaviour was attributed to a decreased intermolecular hydrogen bonding in the crystal lattice, achieved by blocking the 1-NH group by

TABLE 11
Half-times ($t_{½}$) of the Conversion of Various *N*-Acyl Derivatives of Allopurinol (45 – 50) to the Parent Compound at 37°C, Water-solubilities (*S*) and Partition Coefficients (*P*)[a]

Compound	$t_{½}$ (minutes)		*S* (mg/ml at 22°C)	log *P*[b]
	pH 7.4 buffer	80% human plasma		
Allopurinol			0.50	−0.55
1-(Acetyl)allopurinol (45)	26	6	0.75	−0.35
1-(Propionyl)allopurinol (46)	30	4	0.30	0.30
1-(Butyryl)allopurinol (47)	36	2.5	0.11	0.85
1-(Benzoyl)allopurinol (48)	20	4	0.014	1.20
1-(*N*,*N*-Dimethylglycyl)allopurinol HCl (49)	1.5	–	> 100	–
1-(4-*N*,*N*-dimethylaminobutyryl)allopurinol, HCl (50)	< 3 sec	–	> 100	–

a. From Bundgaard and Falch [196].
b. Partition coefficients between octanol and water at 22°C.

acylation. It was suggested that *N*-acylation may be a promising means of obtaining prodrug forms of allopurinol with the aim of enhancing the rectal absorption of the drug [196]. Allopurinol is only very slightly absorbed (< 5%) upon rectal administration [197 – 199], and this is most probably due to its low lipophilicity and poor aqueous solubility. Preliminary experiments in rabbits showed that rectal administration of the N_1-acetyl and N_1-butyryl derivatives in the form of fatty acid suppositories resulted in a bioavailability of allopurinol of 20 – 30% whereas administration of allopurinol itself led to a bioavailability of less than 2% [174, 196]. The N_1-acyl derivatives 49 and 50 were very soluble (> 10% w/v) in water as hydrochloride salts, but due to a very limited solution stability they appear to be less suitable as parenteral prodrug delivery forms. As referred to earlier, more useful allopurinol prodrugs for parenteral administration are *N*-acyloxymethyl derivatives in which the acyl moiety contains an ionizable amino function [172].

Bioreversible derivatization of amino groups by coupling with γ-glutamic acid has received considerable attention. It has been shown that the kidney is highly active in the uptake and metabolism of γ-glutamyl derivatives of amino acids and peptides [200, 201]. This property could be attributed to the high concentration in the kidney of γ-glutamyl transpeptidase, an enzyme capable of cleaving γ-glutamyl derivatives of amino acids and other compounds containing an amino function. Based on this finding, the possibility of using γ-glutamyl derivatives of pharmacologically active substances as kidney-specific prodrugs has been explored by various groups. Thus, Wilk and co-workers [202 – 204] and others [205, 206] have developed γ-glutamyl

derivatives of dopamine and L-dopa as kidney-specific prodrugs. Administration of the derivatives into animals led to a selective generation of the active dopamine in the kidney as a consequence of the high activity of γ-glutamyl transpeptidase in this organ. The γ-glutamyl-L-dopa derivative is initially converted to L-dopa, which then decarboxylates to dopamine by the action of L-amino acid decarboxylase, an enzyme that is also highly concentrated in the kidney (Fig. 3). Tissues other than the kidney showed only low free drug concentrations, resulting in a separation of the renal dopaminergic from the systemic adrenergic effects with the prodrugs. The results suggest that these prodrugs of L-dopa and dopamine may be useful as specific renal vasodilators.

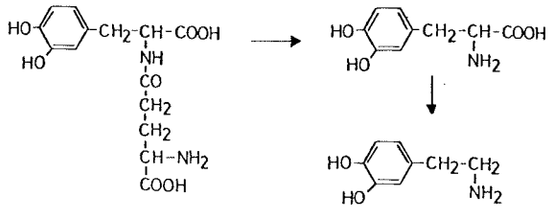
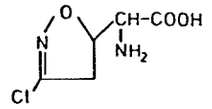


Fig. 3. Selective generation of dopamine in the kidney by sequential action of γ-glutamyl transpeptidase and aromatic L-amino acid decarboxylase on γ-glutamyl dopa.

Similarly, Orlowski et al. [207] have shown that it is possible to obtain a kidney-selective accumulation of sulphamethoxazole by administering the drug in the form of *N*-acyl-γ-glutamylsulphamethoxazole. Besides being dependent on γ-glutamyl transpeptidase, the release of sulphamethoxazole from this derivative requires the action of an *N*-acylamino acid deacylase, which is also present in the kidney in high concentrations.

These findings indicate that γ-glutamyl or *N*-acyl-γ-glutamyl prodrug derivatives of a variety of drugs may be of general use when it is a desired objective to confine the drug action to the kidney and urinary tract. However, a prerequisite for the applicability of this concept is that the γ-glutamyl derivative of a given drug substance can function as a substrate for γ-glutamyl transpeptidase. Recent data provided by Mangan et al. [208] have shown that a great variability exists in the rates of cleavage of γ-glutamyl derivatives of various amino acids and other compounds containing an amino group. Thus, derivatives of 4-aminobutyric acid and L-thiazolidine-4-carboxylic acid as well as of adamantine are not hydrolyzed readily by the enzyme [209].

Plasmin is a protease which cleaves a variety of low-molecular-weight peptides and which is formed in increased amounts in human neoplasms [210]. Recently, Chakravarty and co-workers [211 – 213] have attempted to exploit this feature of malignant cells by preparing peptidyl prodrug derivatives of anticancer drugs that would be activated locally by tumour-associated plasmin. The antimetabolite acivicin (51), the alkylating phenylenediamine mustard (52) and doxorubicin were



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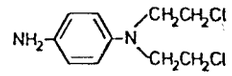
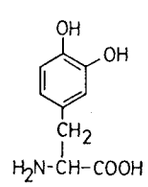


52

derivatized at their free amino group with the tripeptide D-Val-Leu-Lys, to give prodrugs which showed improved selectivity in vitro compared to the parent drugs when tested against transformed and normal chicken embryo fibroblasts. In fact, this improved selectivity was found to be due to the selective activation of the prodrugs by plasmin in the transformed cell cultures.



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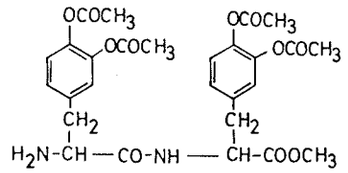

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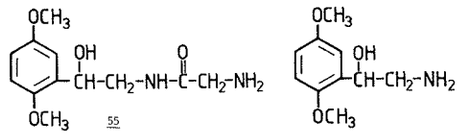
The relatively poor bioavailability of orally administered L-dopa (53) has been attributed to low water and lipid solubility of the compound and to its high susceptibility to chemical and enzymatic degradation, resulting in pronounced metabolic degradation prior to and/or during absorption. Potentially useful prodrugs resulting in improved absorption characteristics of L-dopa have been shown to be various amides or dipeptides, including the combined dipeptide-ester-type structure (54) [214]. The results obtained with L-dopa may imply that dipeptides or amides prepared with amino acids can be used as prodrugs to enhance the oral absorption.

Other enzymatically labile amides or peptides include a *N*-glycyl derivative (55) of midodrin (56) [215] and various amino acid derivatives (57) of benzocaine [216, 217]. The latter compounds are highly water-soluble and are rapidly hydrolyzed in the presence of human serum [218, 219]. Similarly, Eckert et al. [220] have proposed *n*-propylaminoacetylation as a means of obtaining water-soluble bioreversible derivates of aromatic amines, e.g., sulphonamides (Scheme 9). Biel et al. [221] have reported that the *N*-L-isoleucyl derivative of dopamine is cleaved readily by aminoacylarylamidase, an enzyme which is particularly abundant in renal tissue.

A new concept of obtaining site-specific and sustained release of amine drugs to the brain, involving *N*-acylation, has been developed recently by Bodor and co-workers [222, 223]. Because of being highly protonated at physiological pH several amine drugs are not sufficiently lipophilic to be able to pass the blood-brain barrier. By linking such drugs to a lipophilic dihydropyridine carrier through an amide linkage the derivative obtained [D-DHC] (Scheme 10) is distributed quickly throughout the body, including the brain, following its administration. The dihydro form [D-DHC] is then rapidly oxidized enzymatically (by the NAD ⇌ NADH



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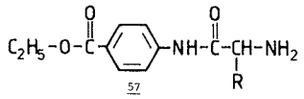

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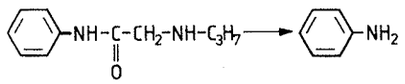
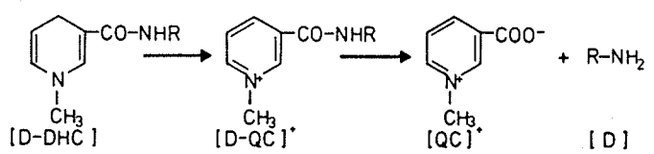


Scheme 9

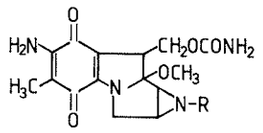
system) to the quaternary salt, $[D\text{-}QC]^+$, which because of its ionic character is eliminated rapidly from the body, except from the brain. Slow enzymatic cleavage of the amide linkage of this compound, locked in the brain, then results in a sustained delivery of the drug species [D] in the brain. This concept has been applied successfully to phenethylamine [224] and dopamine [225] as well as to testosterone [226]. In the latter case the drug release in the brain is due to ester hydrolysis from the quaternary salt.



Scheme 10

N-Acylation of the weakly basic ($pK_a \approx 1.2$) [227, 228] aziridine group in mitomycin C (58) recently has been utilized as the basis for the development of lipophilic prodrugs. Sasaki et al. [229–231] have prepared benzoyl, phenacetyl, benzyloxycarbonyl and various other alkoxycarbonyl derivatives (59–61) and studied their stability in aqueous solution and various biological media. All derivatives hydrolyzed to the parent drug and showed enzyme-mediated conversion in plasma from various species and in rat liver homogenates, to varying extents. The amide derivatives (59) were not converted by plasma enzymes but only by hepatic enzymes, in contrast to the alkoxycarbonyl derivatives (carbamates). The latter derivatives (60) showed the best properties as regards chemical stability and biological lability. The melting points of the derivatives (except for the benzoyl

derivative) were considerably lower than that of mitomycin C and, therefore, the much higher lipophilicity possessed by the derivatives was accompanied by only a relatively slight decrease in water-solubility as compared with mitomycin C. As a consequence of these delivery-improved physicochemical properties the derivatives could be incorporated into lipid carrier systems such as liposomes and o/w emulsions (in contrast to the parent drug), and several of them showed considerable antitumour activations when administered to mice in these lipoidal delivery systems [232]. In addition, the lipophilic prodrugs were described to exhibit enhanced percutaneous absorption, and accordingly they may be useful for topical administration [232].



58 R = -H

59 R = -C(=O)-R′

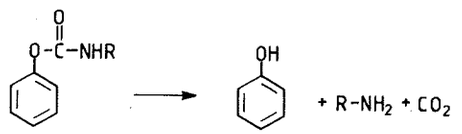
60 R = -C(=O)-OR′

61 R = -CH$_2$OC(=O)-C$_6$H$_5$

Diverse reports in the literature show that sometimes simple amides or carbamates may be applied as prodrugs for amines. Thus, Galzigna et al. [233] reported that the benzoyl or pivaloyl derivatives of γ-aminobutyric acid are hydrolyzed in the presence of rat brain homogenates and that the compounds, by having enhanced capacity to penetrate the blood-brain barrier, should be evaluated as prodrugs.

N-Acetyl-L-cysteine is presently the drug of choice for the clinical treatment of and protection against paracetamol-induced hepatotoxicity. The compound is enzymatically deacetylated in vivo and it most likely acts as a prodrug form of cysteine by releasing the latter in the liver as a biosynthetic precursor of glutathione [234, 235].

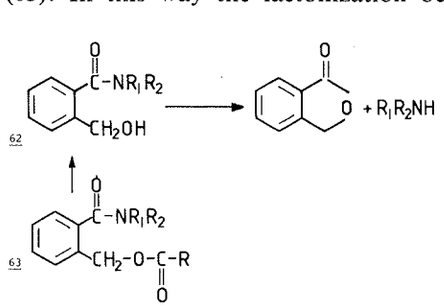
Various carbamate derivatives have been assessed as prodrugs for normeperidine, amphetamine, ephedrine and phenethylamine, but with limited success [236, 237]. Unfortunately, kinetic data on their conversion in aqueous solution and in the presence of plasma or enzymes are not available. As reported by Digenis and Swintosky [6], carbamate esters of phenol are cleaved very rapidly by plasma enzymes (Scheme 11) and, although these authors only classified such structures as possible prodrugs for phenols, they can equally well be considered as prodrug candidates for amines. In such a case, the phenol would be the transport group. Studies along this



Scheme 11

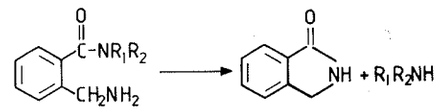
line are certainly warranted since there is a paucity of broadly applicable derivatives for the amino group.

A promising approach to obtaining an amide prodrug capable of releasing the parent amine drug at physiological conditions of pH and temperature is to make use of intramolecular chemical catalysis or assistance of the amide hydrolysis. Thus, 2-hydroxymethylbenzamides (62) undergo a relatively rapid cyclization in aqueous solution, to give phthalide and free amine [238 – 240]. For prodrug design the rate of lactonization is probably too low at pH 7.4, although only amides of ammonia, methylamine and benzylamine have been studied. However, by substituting the two methylene hydrogen atoms of 2-hydroxymethylbenzamide with phenyl and methyl groups greatly increased rate constants for the hydrogen ion-, hydroxide ion- and general base-catalyzed lactonization have been observed [240]. Further steric acceleration of the lactonization may possibly be achieved by the introduction of substituents positioned *ortho* to either the hydroxymethyl group or the carboxamide group. In fact, the pH-independent cyclization of 3-amino-2-hydroxymethylbenzamide proceeds at a rate 10^3 times greater than that of unsubstituted 2-hydroxymethylbenzamide [241]. As suggested by Cain [242, 243], this prodrug principle may become even more attractive by masking the hydroxyl function in the 2-hydroxymethylbenzamides by acylation to give stable 2-acyloxymethylbenzamides (63). In this way the lactonization becomes blocked and must be preceded by



hydrolysis of the ester grouping, i.e., by the action of esterases in vivo. Besides providing an in vitro stable derivative, such cascade latentiation allows the prodrug designer to vary and control the lipophilicity/hydrophilicity of the prodrug by the appropriate selection of the acyl group. While Cain [243] has reported the synthesis of acetyl and benzoyl esters of 2-hydroxymethylbenzamide, information on the stability and enzymatic hydrolysis of such derivatives are not as yet available but are certainly warranted. Studies along this line are being performed currently in this laboratory.

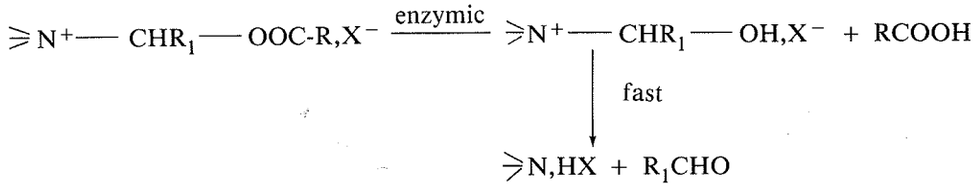
An amino group may also be able to facilitate the cleavage of an amide by intramolecular assistance. Thus, 2-aminomethylbenzamide undergoes a rapid intramolecular aminolysis in aqueous solution to phthalimidine and ammonia (Scheme 12) [244].



Scheme 12

4.2. *N*-Acyloxyalkyl derivatives

N-Acyloxyalkylation of simple primary and secondary amines is not useful because of the extreme lability of such derivatives in aqueous solution [245 – 247]. Sasaki et al. [229, 230] have recently prepared the *N*-benzoyloxymethyl derivative of mitomycin C (61) and shown that it is easily hydrolyzed in aqueous buffer solutions, the half-life being 3.5 minutes at pH 7.4 and 37°C. This relatively high stability may be due to the very low basicity of the aziridine NH-group ($pK_a \approx 1.2$) [227, 228]. For tertiary amines, on the other hand, *N*-acyloxyalkylation results in the formation of chemically stable compounds (quaternary ammonium salts). Due to a high susceptibility to undergo enzymatic hydrolysis (Scheme 13), these compounds (so-called soft quaternary salts) are useful as prodrugs for various tertiary or *N*-heterocyclic amines, as described by Bodor [10, 155, 248] and others [249, 250]. For example, the concept has been applied to improve the ocular delivery characteristics of pilocarpine through long-chain-containing soft quaternary salts [248, 251].



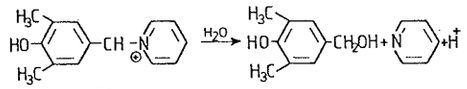
Scheme 13

N-Hydroxymethyl derivatives of amines are more unstable than those of amides or imides. For amines with pK_a values > 2 the half-lives of hydrolysis of such derivatives at neutral pH are less than a few minutes [252].

4.3. Quaternary derivatives of tertiary amines

Besides the quaternary *N*-acyloxyalkyl derivatives mentioned above, compounds of the type 64 may be useful in prodrug design for tertiary amines. Bogardus and Higuchi [253] have shown that *N*-(4-hydroxy-3,5-dimethylbenzyl)pyridinium brom-

ide (64) and similar quaternary derivatives of nicotinamide, *N,N*-dimethylaniline and trimethylamine are cleaved rapidly in neutral and alkaline aqueous solutions, to form the corresponding tertiary amine and 4-hydroxymethyl-2,6-dimethylphenol (Scheme 14). The hydrolysis of these derivatives was shown to proceed through an unstable quinone methide intermediate. Esterification of the phenolic group in structure 64 greatly stabilized the derivatives, and the release of the parent tertiary amine becomes solely dependent on ester hydrolysis. For prodrug design this is highly desirable, in that derivatives are obtained possessing at the same time adequate in vitro stability and susceptibility to undergo rapid enzymatic cleavage in vivo.



64

Scheme 14

4.4. *N*-MANNICH BASES

Besides being considered as a possible approach of derivatizing amide-type compounds (see section 3.1), *N*-Mannich base formation can also be thought of as a means of forming prodrugs of primary and secondary amines, in which case the amide-type component would act as a transport group. As can be seen from Table 12, *N*-Mannich base formation lowers the pK_a values of the conjugate acids of amines by up to about 3 units. Therefore, a potentially useful purpose for transforming amino compounds into *N*-Mannich base transport forms would be to increase the lipophilicity of the parent amines at physiological pH values by depressing their protonation, resulting in enhanced biomembrane-passage properties. This expectation of increased lipophilicity has been confirmed for, e.g., the *N*-Mannich base derived from benzamide and phenylpropanolamine. The partition coefficient of the Mannich base between octanol and phosphate buffer, pH 7.4, was found to be almost 100-times greater than that of the parent amine [132]. By the benzamidomethylation the pK_a of phenylpropanolamine is decreased from 9.4 to 6.15, and the decreased extent of ionization at pH 7.4 is obviously the major contributing factor to the increased lipophilicity of the *N*-Mannich base derivative.

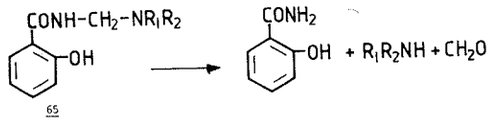
However, the selection of biologically acceptable amide-type transport groups affording an appropriate cleavage rate of a Mannich base of a given amine at pH 7.4 is restricted. In a search for generally useful candidates it was observed [128] that

TABLE 12
Effect of *N*-Mannich Base Formation of Amines on pK_a for the Corresponding Protonated Species[a]

Amine	pK_a value of amine	pK_a value of *N*-benzamidomethylated amine
Piperidine	11.1	7.8
Methylamine	10.7	7.5
Dimethylamine	10.7	7.6
Ethylamine	10.7	7.5
Diethylamine	10.9	7.7
Isobutylamine	10.7	7.5
Cyclohexylamine	10.7	7.6
Benzylamine	9.3	6.4
Morpholine	8.3	5.6
(−)-Ephedrine	9.7	6.3
Phenylpropanolamine	9.4	6.2
Phenethylamine	9.8	7.1

a. From Bundgaard and Johansen [124, 125, 132].

N-Mannich bases (65) of salicylamide and different aliphatic amines (Scheme 15) including amino acids showed an unexpectedly high cleavage rate at neutral pH (cf. Table 4), thus suggesting the utility of salicylamide. The salicylamide Mannich bases possess lower pK_a values than the corresponding benzamide derivatives and the difference in reacitivity may, at least in part, be a reflection of this difference in basicity. Interestingly, the hydrolysis of the salicylamide Mannich bases showed bell-shaped pH-rate profiles with maximum rates at pH around 7; an example is shown in Figure 4.



65

Scheme 15

For aromatic amines, more acidic amide-type transport groups such as succinimide or hydantoins have been suggested in order to ensure a rapid conversion at physiological pH [129], cf. Table 4. A potential objective for transient derivatizing aromatic amino groups in drugs may be to obtain protection against first-pass metabolism by *N*-acetylation [129].

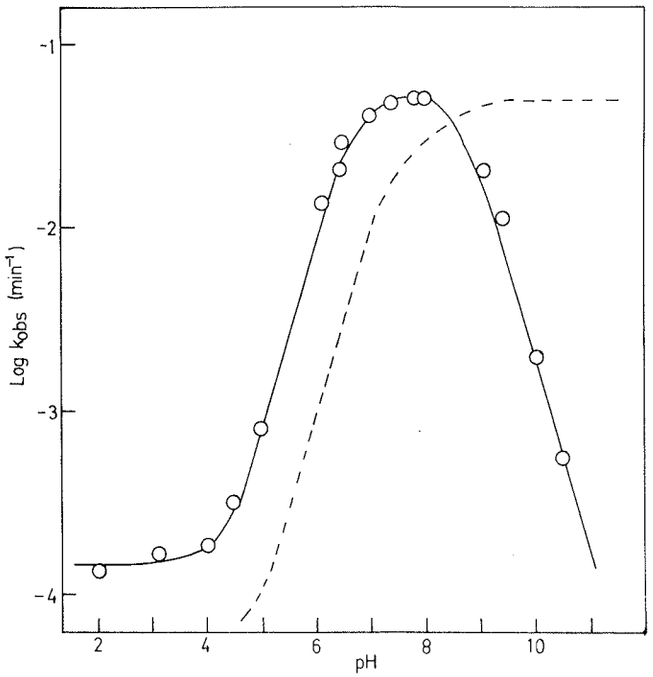
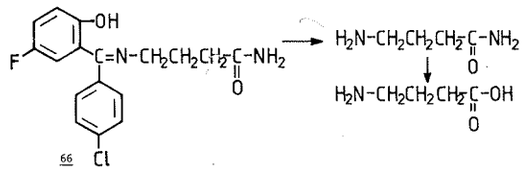


Fig. 4. The pH-rate profiles for the decomposition of *N*-(piperidinomethyl)salicylamide (——) and *N*-(piperidinomethyl)benzamide (– – –) in aqueous solution at 37°C. From Johansen and Bundgaard [128].

4.5. SCHIFF BASES

Schiff bases (imines) may in some particular cases be potentially useful as prodrug derivatives for primary or secondary amines. Their usefulness generally is limited, due to a facile hydrolysis in aqueous solution (see, e.g., Refs. 254, 255). By imine formation the basic character of amines is greatly depressed and, accordingly, Schiff bases are less protonated at physiological pH than are the parent amines. Therefore, a potentially useful purpose of making Schiff base prodrugs would be to increase the lipophilicity of an amine drug, and hence its membrane penetrating ability. Thus, in order to facilitate the passage of γ-aminobutyric acid or its amide derivative across the blood-brain barrier Schiff bases derived from a substituted benzophenone have been investigated [256]. The derivative formed with γ-aminobutyramide, progabide (66), is currently undergoing clinical testing. Besides depression of the pK_a value of the amino group of the parent agent the lipophilicity of this Schiff base prodrug is increased greatly, relative to γ-aminobutyramide, by the two phenyl groups of the pro-moiety. Unfortunately, no kinetic data for the hydrolysis of progabide have apparently been published. The stability may possibly

be rather high since the compound shows some pharmacological effects by itself, e.g., anticonvulsant action [257] and, thus, it is not a true prodrug. Worms et al. [257] and others [258] have examined the pharmacokinetics and metabolic properties of the compound in rats. Extensive metabolism occurs and both γ-aminobutyramide and γ-aminobutyric acid are formed in the brain (Scheme 16).



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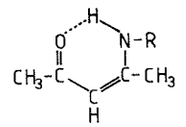
Scheme 16

Other Schiff bases investigated as potential prodrug forms of amines include various derivatives of antimalarial and antileprotic sulphanilylanilides [259] and benzylidene derivatives of sulphamethoxydiazine [260].

4.6. ENAMINONES

Enamines, or α,β-unsaturated amines, are, like most Schiff bases, highly unstable in aqueous solution [260a, 261]. However, enamines of β-dicarbonyl compounds (enaminones) are stabilized relative to the enamines of monocarbonyl compounds, probably due to intramolecular hydrogen bonding, as depicted in structure 67, derived from acetylacetone, and such derivatives may be generally useful prodrugs of amines.

Caldwell et al. [262] have prepared five enaminone derivatives of phenylpropanolamine and shown that the compounds hydrolyzed at vastly different rates in



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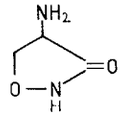

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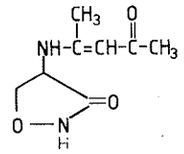

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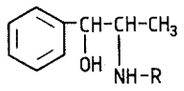
aqueous solution of pH 7.4 (Table 13). The rate of hydrolysis of these compounds increased with decreasing pH. As could be expected, human plasma did not accelerate the rates of hydrolysis. The potential utility of enaminones as prodrugs for amines is stressed further by the study by Dixon and Greenhill [263], who showed that enaminones derived from various 1,3-diketones and a keto-ester had a wide range of hydrolysis rates. More recently, Jensen et al. [264] prepared an enaminone derivative (68) of cycloserine (69), by condensing this with acetylacetone, and showed it to be a potentially useful prodrug with the aim of stabilizing the parent antibiotic which is susceptible to undergo dimerization in the solid state or in solution. Compound 68 was significantly more stable to heat and humidity than was cycloserine, and it exhibited similar antibacterial activity in vitro as the parent drug and gave even superior urinary recovery of free cycloserine upon oral administration to mice [264].

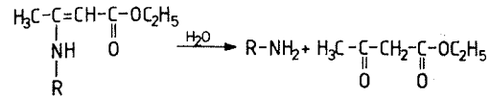
The hydrolysis of enaminones (Scheme 17) derived from ethyl acetoacetate and various amino acids or β-lactam antibiotics with a free amino group has recently been studied by a Japanese group [265, 266]. As appears from Table 14, the derivatives show promise as prodrugs as their half-lives of hydrolysis at physiological pH are fairly rapid. As was shown for the β-lactam antibiotics, the

TABLE 13
Half-Lives of Hydrolysis of Various Enaminones of Phenylpropanolamine at 37°C[a]



R	$t_{1/2}$ (minutes)	
	pH 7.4 buffer	2% human plasma (pH 7.4)
$-C(CH_3)=CH-CON(C_2H_5)_2$	3.4	4.0
$-C(CH_3)=CH-COOC(CH_3)_3$	10.3	9.8
$-C(CH_3)=CH-COOC_2H_5$	40	36
$-CH=C(COOC_2H_5)_2$	stable	stable
$-C(CH_2-COOC_2H_5)=CH-COOC_2H_5$	> 160	160

a. From Caldwell et al. [262].



Scheme 17

TABLE 14
Half-Lives of Hydrolysis of Enaminones Derived from Various Amines and Ethyl Acetoacetate[a]

Amino compound	$t_{1/2}$ at pH 7.4 and 25°C (minutes)
4-Aminobutyric acid	19
Glycine	4
L-Leucine	7
L-Lysine	23
L-Phenylalanine	16
D-Phenylglycine	26
L-Tryptophan	7
Taurine	24
Ampicillin	69
Amoxicillin	79
Cephalexin	98

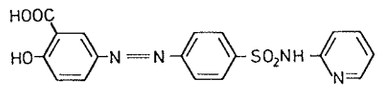
a. From Murakami et al. [265, 266].

enaminone derivatives were much more lipophilic at pH 7.4 than were the corresponding parent drugs [265]. The use of ethyl acetoacetate as the pro-moiety of enaminone prodrugs may possibly also be attractive due to its approval as a food additive and its wide use as a flavouring agent. In accord with their lipophilicity, the enaminones of amino-penicillins and of amino acids such as L- or D-phenylalanine or D-phenylglycine showed markedly improved absorption relative to the parent agents, following rectal administration in rabbits or rats. Interestingly, however, they also promoted the rectal absorption of other compounds, including various polar cephalosporins and insulin [265–269]. The mechanism of this adjuvant effect is not clear as yet, but probably may involve a change of the permeability of the rectal mucosal membrane [269, 270].

On the basis of the studies cited above, enaminones certainly warrant greater attention in the future as a potentially useful bioreversible derivative type for drugs containing a free amino group. In order to be able to apply this promising prodrug approach broadly and rationally, more information is needed on the kinetics and mechanism of enaminone hydrolysis as well as on the relationship between chemical reactivity and structure, involving both the 1,3-dione component and the amino compound.

4.7. AZO COMPOUNDS

Prodrugs containing an azo linkage which is cleaved by azo-reductase enzymes have been used to some extent in drug therapy in order to achieve site-specific drug delivery through selective prodrug activation at the target. An example concerns 5-aminosalicylic acid. Sulphasalazine (70) has long been a widely used drug for the treatment of ulcerative colitis. When the compound reaches the colon after oral administration, the azo bond is split by azo-reductases from colonic bacteria, resulting in the formation of 5-aminosalicylic acid (71) and sulphapyridine [217]. 5-Aminosalicylic acid is the active part of sulphasalazine [272–274] but, if administered as such, it will undergo extensive absorption during its passage through the gastrointestinal tract [275, 276] and not reach its target (the colon) in effective concentrations. However, one serious disadvantage of sulphasalazine is its side effects, which occur in up to 20% of patients taking the drug. These effects are mostly due to the transport group, sulphapyridine [277], which after being released in the colon is absorbed [271].



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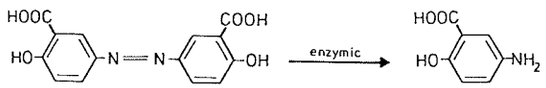
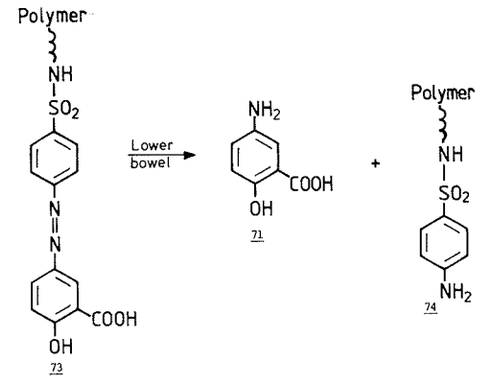


72 71

Thus, it appears that the ideal prodrug would be a derivative that, on the one hand, protects 5-aminosalicylic acid from being absorbed through the gastrointestinal tract and liberates it in the colon but, on the other, lacks the sulphonamide part or other carrier groups, giving rise to unwanted effects. Such an ideal prodrug may be azodisal sodium (72), which simply contains two molecules of 5-aminosalicylic acid linked together by a site-specific cleavageable azo bond. Recent investigations [278, 279] have shown that this derivative, which may be called an 'identical twin', passes through the small intestine with a minimum of absorption or degradation, and when reaching the colon the azo bond is split, with the release of two molecules of the active 5-aminosalicylic acid.

Other promising 5-aminosalicylic acid prodrugs based on bioactivation through azo-reductases in the colon are salicylazobenzoic acid [280] and a water-soluble polymer (73) [281]. Based on experiments in rats, the potential therapeutic advantages of this polymeric prodrug, which contains sodium 5-aminosalicylate residues linked at the 5-position by an azo bond to an inert polysulphanilamide backbone (74), were suggested to include non-absorption/non-metabolism in the small intestine, direct 5-aminosalicylic acid release (Scheme 18) at the diseased site in the lower bowel, and non-absorption/non-metabolism of the released carrier polymer.

Other prodrugs activated by azo-reductases include various azobenzene mustards thought to be cleaved to the parent reactive amines by azo-reductases present in malignant hepatocytes [282, 283]. Although normal liver cells also have the azo-reductase enzymes, they will be less susceptible to the toxicity of the parent alkylating agent, as they are not in cycle.



73 71 74

Scheme 18

4.8. OXAZOLIDINES AND 4-IMIDAZOLIDINONES

A description of oxazolidines as a potential prodrug type for β-aminoalcohols and of 4-imidazolidinones as a prodrug type for the α-aminoamide moiety occurring in, e.g., several peptides is given in sections 7.3 and 7.5, respectively.

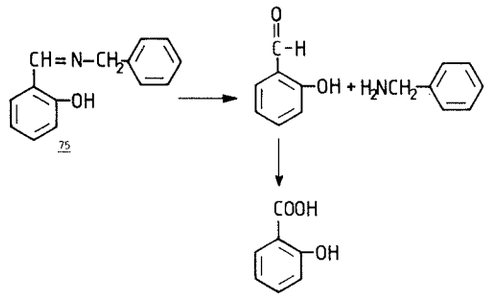
5. Prodrugs for compounds containing carbonyl groups

Only few bioreversible derivatives have been explored of molecules containing an aldehydic or ketonic functional group, but in view of the fairly large number of drugs (e.g., various steroids) containing a carbonyl group this area of prodrug chemistry is certainly going to attract much interest in the future. Derivatives which can be considered as prodrug candidates include Schiff bases, oximes, oxazolidines, thiazolidines and enol esters.

5.1. SCHIFF BASES AND OXIMES

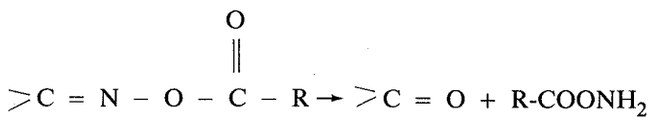
These types of derivative have only found minimal use as prodrug forms. As men-

tioned in section 4.5, Schiff bases have largely been used as prodrug forms for amino compounds. An example of Schiff base formation of a carbonyl-containing drug is *N*-(2-hydroxyphenylmethylidine)benzylamine (75). This compound (also called saddamine) has been described as a prodrug of salicylic acid [284, 285]. It is well absorbed upon oral administration and is cleaved hydrolytically to benzylamine and salicylaldehyde, the latter being oxidized rapidly in vivo to salicylic acid (Scheme 19). The Schiff base derivative is probably already hydrolyzed in the gastrointestinal tract before absorption and it is difficult to imagine a significant therapeutic advantage by this prodrug.



Scheme 19

Mitra et al. [286] have prepared the oxime of norethindrone at its 3-keto group and coupled it to polyglutamic acid by esterification. When dissolved in ethanol/water at pH 6 the polymeric compound slowly hydrolyzed, with formation of the parent drug. The release of norethindrone showed apparent zero-order kinetics and, accordingly, the polymeric prodrug system may allow a constant drug release. It was found further by studying the *O*-acetyl oxime of norethindrone that acylation of the oxime caused a marked enhancement in the rate of hydrolysis of the oximino bond. On this basis it probably may be worth investigating the possibility of using *O*-acylated oximes as prodrug forms for carbonyl-containing substances. Whereas oximes result in the formation of hydroxylamine which may be somewhat toxic, hydrolysis of *O*-acylated oximes results in the formation of a carbamate (Scheme 20).

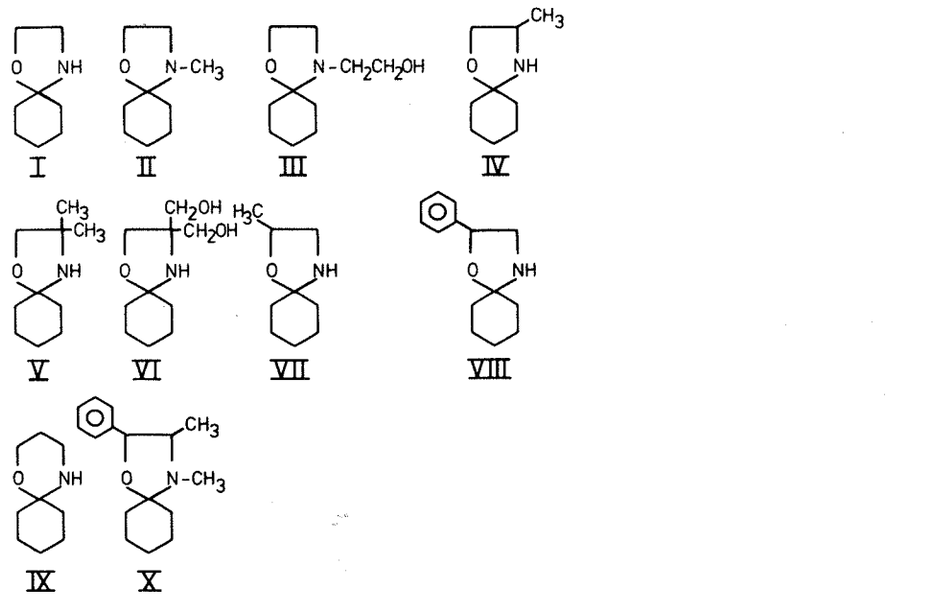


Scheme 20

5.2. OXAZOLIDINES AND THIAZOLIDINES

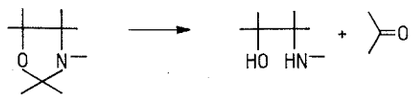
Besides being considered as a possible approach to derivatizing β-aminoalcohols (see section 7.3), oxazolidine formation can also be thought of as a means of forming prodrugs of aldehydes or ketones, in which case the β-aminoalcohol component would act as a transport group (Scheme 21). As described in section 7.3, a study

TABLE 15
Half-lives of Overall Hydrolysis of Various Oxazolidines of Cyclohexanone in Acidic and Neutral Aqueous Solutions at 37°C[a]



Oxazolidine	$t_{1/2}$ (minutes)	
	pH 2.0	pH 7.0
I	3.8	0.07
II	3.1	0.2
III	5.8	0.3
IV	14.4	0.4
V	12.6	42
VI	77.9	158
VII	6.3	0.2
VIII	5.0	0.2
X	230	5.3

a. From Buur and Bundgaard [287].

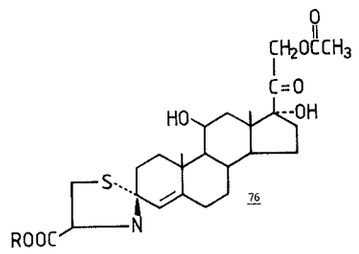


Scheme 21

[287] of the hydrolysis kinetics of several oxazolidines derived from cyclohexanone (a model of a carbonyl-containing drug) and various β-aminoalcohols has provided basic information on the structural factors within the β-aminoalcohol moiety which may influence the stability and reactivity of oxazolidines. It appears from this study that by appropriate selection of the β-aminocarbonyl moiety it is feasible to obtain oxazolidine prodrugs of carbonyl compounds with greatly varying rates of hydrolysis at physiological conditions of pH and temperature (Table 15). In addition, it is possible to confer varying degrees of lipophilicity or hydrophilicity on the oxazolidines by varying the β-aminoalcohol component. In considering oxazolidines as prodrug candidates for carbonyl-containing substances, their weakly basic character (pK_a 5 – 7) [288] may be advantageous in that the transformation of such substances into oxazolidines introduces a readily ionizable moiety, which may allow the preparation of derivatives with increased aqueous solubilities at acidic pH values. For example, a potentially useful purpose of transforming a carbonyl-containing drug substance into a bioreversible oxazolidine derivative could be to enhance its dissolution behaviour in an effort to improve the oral bioavailability. This aspect is currently being tested in this laboratory with some slightly water-soluble steroids. In the same context, it is also worth mentioning that whereas benzaldehyde is an easily oxidizable liquid with a pungent odour, several oxazolidine derivatives of the compound are crystalline solids, e.g., the derivatives formed with (−)-ephedrine or (+)-pseudoephedrine, which in addition show high solid-state stability [289]. Benzaldehyde recently has attracted considerable attention as a potential anti-tumour agent [290 – 293], but its physical properties create obvious formulation problems. A peroral dosage form of benzaldehyde in the form of a crystalline oxazolidine prodrug may possibly be useful in overcoming such problems. After administration, benzaldehyde will be released by spontaneous hydrolysis in the gastrointestinal tract and/or in the blood after absorption of the prodrug, depending on the stability in the stomach and intestine [289].

In a search for improved topical anti-inflammatory steroids using the prodrug approach, Bodor and co-workers [294 – 296] have shown the applicability of thiazolidines as bioreversible steroidal 3-ketone derivatives. A series of thiazolidine derivatives (spirothiazolidines) of hydrocortisone and hydrocortisone 21-acetate (76) were prepared, using cysteine esters or related β-aminothiols as the derivatizing agents, and were shown to be converted readily to the parent corticosteroids at conditions similar to those prevailing in the skin, thus meeting the requirement for a prodrug. The opening of the thiazolidine ring proceeds by a spontaneous S_N1

cleavage of the carbon-sulphur bond to give a Schiff base intermediate, which then is hydrolyzed. Some of the thiazolidines were found to be more active than the parent steroid in animal studies and, also, to exhibit less systemic toxicity when applied topically [294]. It was speculated that the Schiff base intermediate formed upon ring-opening may accumulate in the skin by binding (through its -SH function) to thiol groups in the skin, thus providing a depot or a slow release form of the active steroid. Cysteine derivatives, in particular, may be attractive as pro-moieties because of the release of cysteine as a by-product. Also, the carboxylic group of cysteine is easily esterifiable, thus providing a convenient method for changing the lipophilicity/hydrophilicity of the spirothiazolidine prodrugs.



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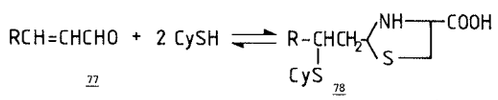
This prodrug approach has also been extended to other steroids, such as testosterone and progesterone [295]. For the latter compound thiazolidines were obtained at both the 3-keto and the 20-keto group.

Thiazolidines derived from cysteine have been proposed as prodrug forms for various α,β-unsaturated aldehydes (e.g., 4-hydroxypentenal and crotonal) with antitumour activity, as a means of prolonging the action and decreasing the toxicity of these aldehydes [297 – 299]. Besides forming a thiazolidine with the aldehyde group, cysteine also adds to the C = C double bond of the α,β-unsaturated aldehydes (77) to give products of the structure 78. These adducts are in aqueous solution in equilibrium with cysteine and the parent aldehydes. The kinetics of the reactions have been studied by Esterbauer et al. [297], and it was shown that the adducts decompose by first splitting off the cysteine residue of the side chain, followed by a more slow hydrolysis of the thiazolidine ring.

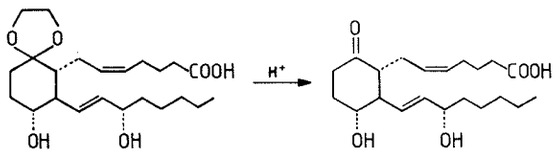


77 78

5.3. Acetals and Ketals

Such prodrug derivatives have found some utility for oral administration since they easily revert to the parent carbonyl-containing compound under acidic conditions. Cho et al. [300] prepared an ethylene ketal of prostaglandin E_2 (dinoprostone) in

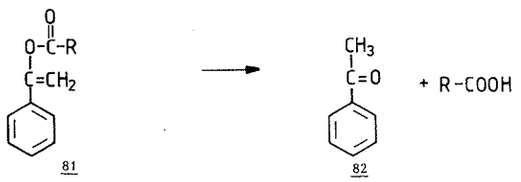
order to improve the chemical stability of this drug. Like most β-hydroxy ketones, the E series prostaglandins readily undergo dehydration to produce the A series prostaglandins, but by saturating the carbonyl group at C-9 of prostaglandin E_2 (79) the dehydration reaction can be depressed. The ethylene ketal derivative (80) was found to possess much improved solid-state stability, and it may be an orally useful prodrug form since it readily undergoes an acid-catalyzed hydrolysis back to the parent prostaglandin under conditions similar to those prevailing in the stomach. Experiments in monkeys showed that the ketal derivative was bioequivalent to the parent drug following oral administration, thus indicating the ready cleavage of the ketal in the stomach [300].



80 79

5.4. Enol esters

Several drugs contain enolizable carbonyl groups as their most prominent functional group (such as some steroids, anticoagulants and phenylbutazone). Although the keto-enol equilibrium usually lies far in favour of the keto form, the enol form can under proper conditions be trapped by alkylation or acylation of the hydroxyl (enol) group. Such enol ethers and esters may undergo ready hydrolysis with liberation of the free enol, which then reverts to the keto form almost instantaneously. Using acetophenone (82) as a model for an enolizable carbonyl-containing drug substance Patel and Repta [301, 302] have demonstrated that enol esters may be quite useful as prodrugs of such agents. The stability of several enol esters of acetophenone (α-acyloxystyrenes) (81) were evaluated in aqueous buffer solutions and in human and rat plasma and liver homogenates. The derivatives behaved much as saturated esters and were relatively stable in aqueous solution, with maximum stability at pH 3 – 5, but most derivatives hydrolyzed rapidly and completely to yield the parent acetophenone (82) with the aid of enzymatic catalysis in plasma or liver homogenate. Some rate data are listed in Table 16. Steric and electronic effects within the acyl group are seen to have a substantial influence upon both the aqueous



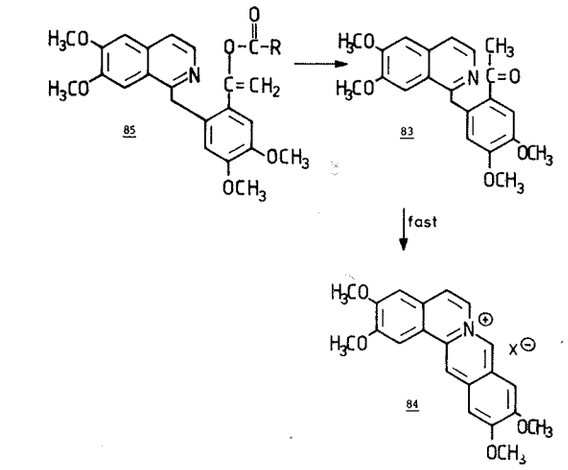
81 82

and enzymatic rates of hydrolysis. It is evident that by appropriate selection of the acyl group it is possible to obtain enol ester prodrugs with varying rates of hydrolysis as well as with varying lipophilicity or aqueous solubility.

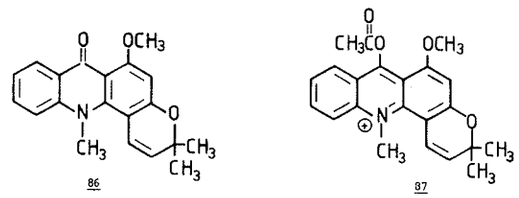
TABLE 16
Half-lives of Hydrolysis of Various α-Acyloxystyrenes (81) at 37°C[a]

R in structure 81	$t_{1/2}$ (minutes)		
	5% human plasma	1% rat plasma	Human liver supernatant
$-CH_3$	26	1.9	6.3
$-CH_2CH_3$	13	0.5	2.8
$-CH_2CH_2CH_3$	12	0.7	1.4
$-CH(CH_3)_2$	110	3.1	4.8
$-C(CH_3)_3$	–	2.0	11.1
$-C_6H_5$	55	1.1	2.0
$-CH_2N(CH_3)_2$	21	2.6	6.6

a. From Patel and Repta [302].



85 83 84

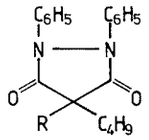


86 87

Based on the results obtained with the model α-acyloxystyrenes Repta and Patel [303] developed enol esters of 6′-acetylpapaverin as potential prodrug forms of the quaternary antitumour agent coralyne, with the aim of delivering this agent to the brain. 6′-Acetylpapaverin (83) itself was developed previously [304] as a lipophilic un-ionized prodrug of coralyne (84), as it readily cyclizes ($t_{1/2} \approx 1$ minute) to coralyne at pH 7.4 and 37°C. By conversion of 6′-acetylpapaverin to an enol ester (85) cyclization is prevented and, in accordance with the results of the model studies, the alkyl esters prepared (85) were found to exhibit adequate stability in aqueous solutions (from a pharmaceutical standpoint) while being easily hydrolyzed in vivo to coralyne via the intermediate 6′-acetylpapaverin as a result of enzymatic catalysis by unspecific esterases [303]. While the 6′-acetylpapaverin prodrug (83) was found to afford an enhanced and sustained delivery of the parent quaternary species coralyne to the brain, enol esters of 6′-acetylpapaverin (85) function as pharmaceutically stable prodrug forms [305]. Thus, this represents an excellent example of the solution of delivery and formulation stability problems by using cascade latentiation or pro-prodrugs.

Acronine (86), an investigational antitumour agent, has such a low aqueous solubility (2 – 3 μg/ml) that intravenous administration of sufficient amounts is impossible. This problem has been overcome by converting the drug to an acylated enol prodrug form. Acetylacroninium perchlorate (87) has been shown [306] to possess greatly enhanced solubility relative to acronin and to be hydrolyzed rapidly ($t_{1/2} \approx 5$ minutes) at pH 7.4 and 37°C. This rate is certainly even higher in vivo due to enzymatic catalysis. Although such rapid hydrolysis is desired following parenteral administration, the instability of the prodrug presents problems in the preparation of the intravenous formulation. Interestingly, these problems may apparently be solved by adding sodium gentisate to the formulation, in that this agent through complex formation with the acetylacroninium ion was found to protect the latter against hydrolysis [307, 308].

A final example of obtaining enol ester prodrug forms of molecules containing an enolizable ketone carbonyl function concerns phenylbutazone (88) and the related oxyphenbutazone. Besides various enol esters (89) [309 – 312], bioreversible *O*-acyloxymethyl derivatives (90) have been described [311, 312]. Although no data



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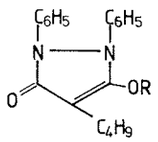


89 R = –C(=O)–R′

90 R = –CH2OC(=O)–R′

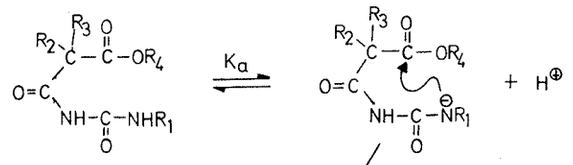
are available, the latter derivatives may certainly be more attractive since these enol ether-esters are expected to be much more stable in vitro than enol esters. The enolic function of phenylbutazone is relatively acidic (pK_a 4.4) [313] and, therefore, the enolate anion (Drug-O^-) should be a good leaving group, making the enol esters highly unstable. In the *O*-acyloxymethyl derivatives the leaving group is the much more basic *O*-hydroxymethyl anion (Drug-OCH_2O^-).

6. Ring-opened derivatives as prodrugs for cyclic drugs

Various ring-opened derivatives of cyclic drugs have been proposed as potentially useful prodrugs with either increased lipid or water solubility. Such cyclic drugs or chemical entities include barbituric acids, hydantoins, 2,4-oxazolidinediones, imides, γ-lactones, cyclic quaternary ammonium compounds and 1,4-benzodiazepines.

6.1. Barbituric acids

Esters of malonuric acids (91) have been shown to be prodrug candidates for their respective barbituric acids (92), including thiobarbituric acids [314 – 316]. The esters were found to undergo a rapid and quantitative cyclization in neutral and alkaline aqueous solution to the corresponding barbituric acids, the rate increasing with increasing pH. The apparent specific base catalysis was interpreted in terms of a mechanism involving intramolecular nucleophilic attack of the terminal ureido nitrogen anion on the ester carbonyl moiety. The rates of cyclization were unaffected by the presence of human blood serum, and a possible enzymatic hydrolysis of the esters to the non-cyclizing free malonuric acids was found to be of no importance as compared with the spontaneous ring-closure reaction [315]. Table 17 contains rate data for the cyclization of a series of malonuric acid esters as well as values for the octanol-water partition coefficients. It is readily apparent that appropriate



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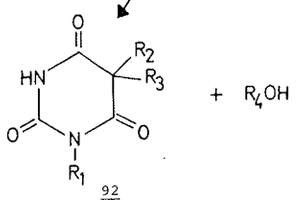

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TABLE 17
Half-lives for Cyclization of Malonuric Acid Esters to Barbituric Acids at 37°C and pH 7.4, and Partition Coefficients of the Esters and Corresponding Barbituric Acids at 23°C[a]

Compound	$t_{½}$ (minutes)	log P[c]
Barbital		0.66
Methyl 2,2-diethylmalonurate	21	0.95
Ethyl 2,2-diethylmalonurate	50	1.45
Isopropyl 2,2-diethylmalonurate	395	1.99
Benzyl 2,2-diethylmalonurate	26	2.74
Methoxymethyl 2,2-diethylmalonurate	14	1.00
Methoxycarbonylmethyl 2,2-diethylmalonurate	11	0.99
Phenobarbital		1.41
Methyl 2-ethyl-2-phenylmalonurate	32	1.58
Ethallobarbital		0.83
Methyl 2-ethyl-2-allylmalonurate	12	1.19
Allobarbital		1.08
Methyl 2,2-diallylmalonurate	8	1.37
Hexobarbital		1.45
Methyl 2-methyl-2-cyclohexenyl-6-methylmalonurate	63	1.75
Ethyl 2-methyl-2-cyclohexenyl-6-methylmalonurate	170	2.25
Methoxymethyl 2-methyl-2-cyclohexenyl-6-methylmalonurate	48	1.80
Thiobarbital[b]		1.57
Methyl diethylthiomalonurate[b]	0.5	1.82

a. From Bundgaard et al. [315], unless otherwise specified.
b. From Bundgaard et al. [316].
c. P is the partition coefficient between octanol and 0.1 M acetate buffer of pH 3.5.

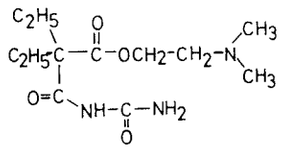
selection of the alcohol moiety of these esters enables one to confer almost any desired degree of lipophilicity or hydrophilicity on the prodrugs and to obtain any desirable rate of the conversion reaction. For the malonuric acid esters the water solubility (S, in molar concentration) and the partition coefficients between octanol and water (P) were related by the expression [315]:

$$\log S = -(0.89 \pm 0.10) \log P - (1.05 \pm 0.17) \quad (8)$$

Because the melting points of the compounds did not differ appreciably, a term for melting point need not be included in this equation. The correlation observed illustrates clearly the important fact in prodrug design that increased lipophilicity normally is accompanied by a decreased (predictable) water solubility, or vice versa (provided melting points are unchanged).

The activity characteristics of barbiturates can be controlled to a great extent by variation in their lipophilicity [317]. In practice, this can be done and has been done by changing the 5-substituents, giving new analogs. However, the same may certainly be achieved by making use of the prodrug concept, and thus permitting the control of the hydrophobic character or other physicochemical properties of a given barbituric acid derivative [315].

By introducing an ionizable group in the alcohol portion of a malonuric acid ester, it is possible to obtain a derivative with greatly increased aqueous solubility. A suitable prodrug candidate may be a *β-N,N*-dimethylaminoethyl ester. Such an ester (93) of barbital was shown to cyclize rapidly to the parent compound, the half-life being 2.8 minutes at pH 7.4 and 37°C [318]. The solubility of the hydrochloride salt of the ester in water was found to be greater than 75% w/v. These results suggest that *β-N,N*-dimethylaminoethyl esters of malonuric acids or thiomalonuric acids may be potentially useful water-soluble prodrugs of the respective barbituric acids, with improved delivery properties for parenteral use.



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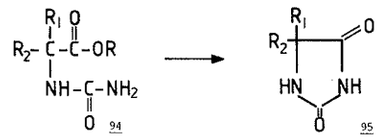
6.2. HYDANTOINS

Stella and co-workers [319, 320] have shown that esters of hydantoic acid (94) may be promising prodrugs for the corresponding hydantoins (95). As is the case for the malonuric acid esters described above, the ring-closure of hydantoic acid esters proceeds readily in neutral and alkaline aqueous solutions, it being subject to apparent hydroxide ion catalysis and requiring no enzymatic mediation. Derivatives with good aqueous solubilities and of potential use as improved parenteral delivery forms for mephenytoin [319] or phenytoin [320] are various salts of the corresponding *β-N,N*-diethylaminoethyl hydantoates. The half-lives of ring closure of these esters were found to be 17 and 7 minutes, respectively. The pharmacokinetics of the phenytoin prodrug in rats has been reported [321].



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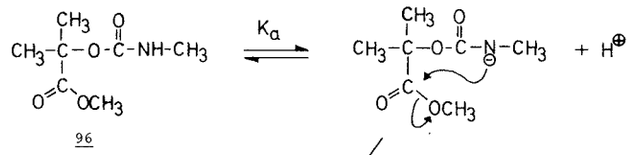
6.3. 2,4-OXAZOLIDINEDIONES

Using the anticonvulsant drug trimethadione as a model substance esters of the ring-opened isobutyric acid derivative have been suggested to be suitable prodrug can-

didates for 2,4-oxazolidinediones [322]. The methyl ester derivative (96) was found to undergo a rapid and quantitative cyclization in aqueous solution to trimethadione (97), the reaction showing apparent specific base catalysis. At pH 7.4 and 37°C the half-life of ring closure is 0.8 minute. The ester (96) is more lipophilic than trimethadione, as determined from octanol-water partition coefficients (the log *P* values being 0.41 and 0.07, respectively), and it was suggested that the prodrug ester or other more lipophilic ester derivative could increase the anticonvulsant activity of the parent drug by facilitating its transport to its receptor site [322].



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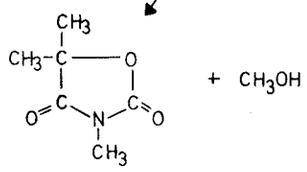

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100 R = CH_3

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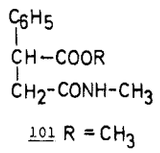
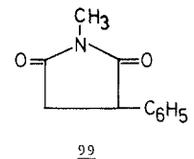


101 R = CH_3



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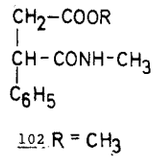


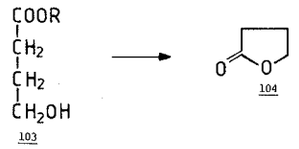
102 R = CH_3

6.4. IMIDES

Using glutethimide (98) and phensuximide (99) as model substances, methyl esters of the ring-opened glutaramic acid (100) and succinamic acid (101 and 102) derivatives were evaluated as possible prodrug types for glutarimides and succinimides, respectively [323]. The esters underwent a quantitative and apparent specific base-catalyzed cyclization in neutral and alkaline aqueous solution, but the conversions were rather slow at physiological pH. The half-lives at pH 7.4 and 37°C were 16.6 hours for methyl 4-ethyl-4-phenylglutaraminate (100) and 2 hours for both methyl *N*-methyl-3-phenylsuccinamate (101) and methyl *N*-methyl-2-phenylsuccinamate (102) [323]. Esters more reactive than the methyl ester, e.g., phenyl esters, may certainly result in increased rates of ring closure, and therefore esterification of glutaramic and succinamic acids may in principle be a method of obtaining prodrug forms of glutarimides and succinimides.

6.5. γ-LACTONES

Esters of the appropriate 4-hydroxybutyric acids have been reported [324] to be potentially useful prodrug forms for the γ-lactone moiety which occurs in several drugs, such as pilocarpine, spironolactone, digoxin, noscapine and canrenone. Various esters of 4-hydroxybutyric acid (103) were shown to cyclize quantitatively to γ-butyrolactone (104) in neutral and alkaline aqueous solution [324]. The rates of lactonization were directly proportional to the hydroxide ion concentration, up to a pH of at least 10, the reactions most likely taking place by an intramolecular nucleophilic attack of alkoxide ion on the ester carbonyl moiety. As appears from the rate data given in Table 18, lactone formation proceeds rather slowly at pH 7.4 for simple alkyl esters but is greatly accelerated in the case of the phenyl ester. Accordingly, such an ester may be a good choice for ensuring a sufficiently rapid formation in vivo of the γ-lactone. It should be noted, however, that steric effects exhibited by various substituents in the hydroxy esters certainly may have an influence on the rate of ring closure. Thus, the cyclization of the methyl ester of 2-methyl-4-hydroxybutyric acid to α-methyl-γ-butyrolactone has been found to proceed with a half-time of 60 minutes at pH 7.4 and 37°C (Bundgaard, unpublished data), which is significantly more rapid than in the case of the unsubstituted hydroxy ester (192 minutes). It is also seen from Table 18 that the 4-hydroxybutyric acid esters are more lipophilic than the parent lactone. This increased lipophilicity may become advantageous in situations where delivery problems for lactone drugs



103 104

TABLE 18
Half-lives for Cyclization of 4-Hydroxybutyric Acid Esters to γ-Butyrolactone at 37°C and pH 7.4, and Partition Coefficients of the Esters and the Parent Compound at 23°C[a]

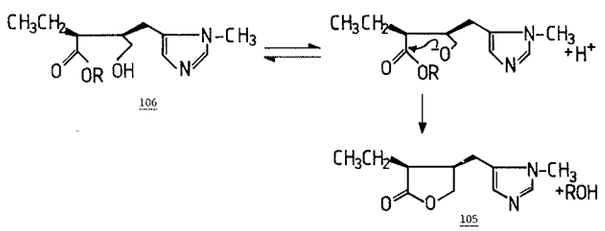
Compound	$t_{½}$	log P[b]
γ-Butyrolactone		−0.31
Methyl 4-hydroxybutyrate	3.2 h	−0.06
Ethyl 4-hydroxybutyrate	13.7 h	0.43
Phenyl 4-hydroxybutyrate	≈ 5 min	1.21

a. From Bundgaard and Larsen [324].
b. P is the partition coefficient between octanol and water.

are due to low lipophilicity. Thus, this is the case for pilocarpine and, recently, the prodrug principle outlined has been applied to this drug.

Although pilocarpine (105) is used widely as a typical miotic for controlling the elevated intraocular pressure associated with glaucoma, the drug presents severe delivery problems. Its ocular bioavailability is very low, which can be ascribed in part to resistance to corneal penetration, and hence to its low lipophilicity [325–327]. Besides, pilocarpine has a short duration of action, thus requiring very frequent administration. Such administration of massive amounts of the compound often results in poor patient compliance, and furthermore is associated with transient peaks of high drug concentration in the eye, which in turn results in undesirable side-effects such as myopia and miosis. These shortcomings of pilocarpine may probably be overcome by the prodrug approach. To be successful a pilocarpine prodrug should exhibit a high lipophilicity in order to enable an efficient penetration through the corneal membrane, should be converted to the active parent drug once the corneal barrier has been passed and, finally, should lead to a controlled release, and hence prolonged duration of action of pilocarpine.

Pilocarpic acid esters may be promising prodrug candidates with these desirable attributes [328]. A series of alkyl and aralkyl esters of pilocarpic acid (106) has been prepared and shown to function as prodrugs of pilocarpine (105) both in vitro and



Scheme 22

TABLE 19
Rate Data for the Conversion of Pilocarpic Acid Esters to Pilocarpine in Aqueous Solution (37°C), and Partition Coefficients for the Compounds[a]

Compound	k_{OH}[b] (M^{-1} min)	$t_{½}$[c] (minutes)	log P[d]
Pilocarpine			−0.15
Pilocarpic acid esters (106), R			
Ethyl (II)	2.25×10^3	510	0.58
Butyl (III)	1.40×10^3	820	1.58
Hexyl (IV)	1.04×10^3	1105	2.56
Benzyl (V)	2.30×10^4	50	1.82
4-Chlorobenzyl (VI)	3.83×10^4	30	2.54
4-Methylbenzyl (VII)	1.49×10^4	77	2.31
4-*tert*-Butylbenzyl (VIII)	1.32×10^4	87	3.52
Phenethyl (IX)	5.07×10^3	227	2.16
2-Methylbenzyl (X)	8.28×10^3	139	2.27
α-Methylbenzyl (XI)	2.42×10^3	475	2.08

a. From Bundgaard et al. [328, 329].
b. Apparent specific base catalytic rate constant.
c. Half-lives of lactonization at pH 7.40.
d. Partition coefficients between octanol and 0.05 M phosphate buffer solution of pH 7.40.

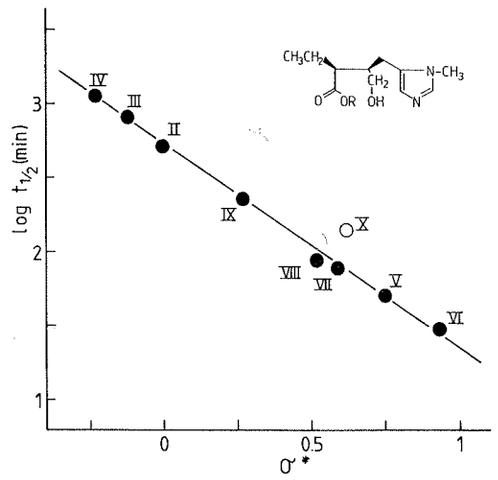


Fig. 5. Semilogarithmic plot of half-lives of conversion of various pilocarpic acid esters (106) to pilocarpine (pH 7.40 and 37°C) against the Taft polar substituent parameter σ*. The latter refers to R in RCH_2OH for the alcohol moieties in the esters. The numbers refer to the compounds in Table 19. From Bundgaard et al. [329].

in vivo [328 – 330]. In aqueous solution the esters undergo a quantitative and apparent specific base-catalyzed lactonization to pilocarpine (Scheme 22). As appears from the rate data obtained (Table 19), the various esters differ greatly in their rates of cyclization. Except for the sterically hindered 2-methylbenzyl and α-methylbenzyl esters, the variation of the rates of lactonization of these ester derivatives could be fully accounted for in terms of polar effects exhibited by the alcohol portions of the esters [329]. The following correlation was found between the half-time (in minutes) of pilocarpine formation from these esters at pH 7.4 and 37°C and the Taft polar substituent parameter σ^*, the latter referring to R in RCH_2OH for the alcohols (Fig. 5):

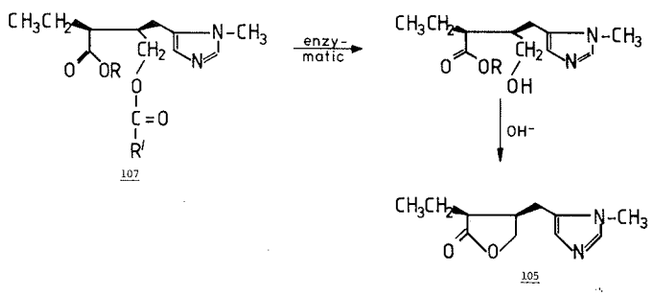
$$\log t_{1/2} = -1.44\,\sigma^* + 2.73 \qquad (n = 8;\ r = 0.998) \tag{9}$$

It is readily evident that by appropriate variation of the alcohol portion of the esters there are ample possibilities to vary and predict the rate of ring closure, and hence to control and modify the rate of pilocarpine generation. Further studies showed that even in the presence of 75% human plasma or rabbit eye tissue homogenates, the cyclization reactions predominated entirely over hydrolysis of the pilocarpic acid esters to pilocarpic acid, which does not cyclize to pilocarpine at physiological pH. The lactonization rates observed in these media were identical to those in pure buffer solutions [329].

In accordance with the predictions made with the γ-butyrolactone/4-hydroxybutyrate ester model system, the pilocarpic acid esters were found to be much more lipophilic than the parent pilocarpine (Table 19). Appropriate selection of the alcohol portion of the pilocarpic acid esters enables one to confer almost any desired degree of lipophilicity on the prodrugs.

Studies in rabbits have confirmed that several of the pilocarpic acid esters give rise to improved ocular bioavailability of the parent drug and, furthermore, result in a more prolonged duration of action of pilocarpine [328, 330].

The main drawback of these pilocarpic acid esters is their limited solution stability, making it difficult to prepare ready-to-use solutions with a not too low pH and



107 105

Scheme 23

possessing an acceptable shelf-life [329]. However, this problem can be overcome totally by blocking the free hydroxy group in the esters by esterification. The double esters (107) thus obtained are highly stable in aqueous solutions, even at pH 5 – 6 (shelf-lives exceeding 5 years at 25°C) and, most significantly, are subject to facile enzymatic hydrolysis at the *O*-acyl bond [328, 330]. It has thus been demonstrated that in the presence of human plasma or rabbit eye tissue homogenates pilocarpine

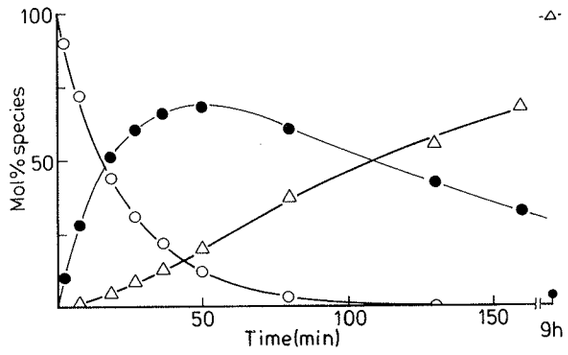


Fig. 6. Time-courses for *O*-benzoyl pilocarpic acid 4-methylbenzyl ester (○), pilocarpic acid 4-methylbenzyl ester (●) and pilocarpine (△) during incubation of the *O*-benzoyl derivative (○) in 75% human plasma (pH 7.4) at 37°C. From Bundgaard et al. [328, 331].

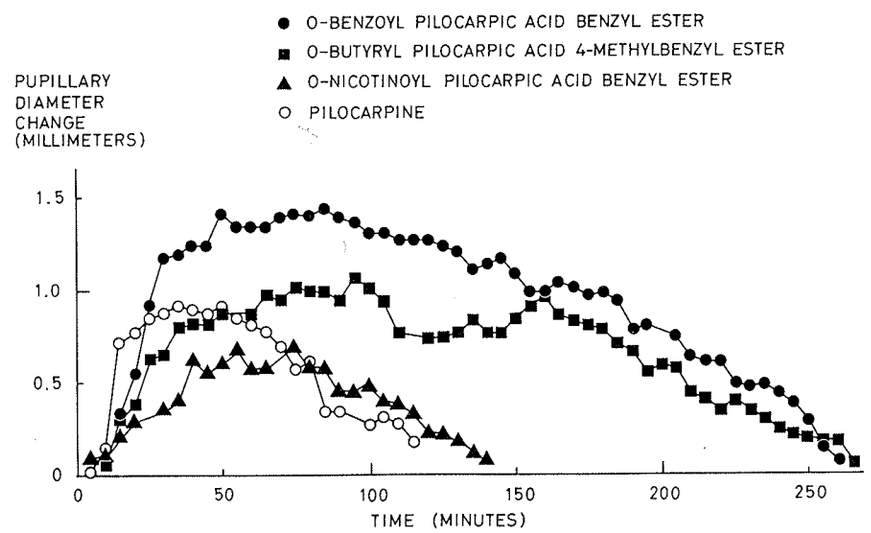
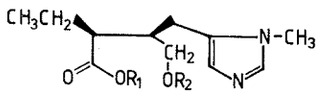


Fig. 7. Plots of the average observed changes in pupillary diameter as a function of time following the instillation of 25 μl of isotonic solution (pH 4.5) in equimolar concentrations (0.25% pilocarpine nitrate equivalent) of the compounds indicated. Four rabbits were used in the crossover study. From Bundgaard et al. [328].

is formed from these derivatives in quantitative amounts through a sequential process involving enzymatic hydrolysis of the *O*-acyl bond, followed by the spontaneous lactonization of the intermediate pilocarpic acid ester (Scheme 23) (Fig. 6). Besides solving the stability problem of the pilocarpic acid esters, the cascade latentiated derivatives *O*-acyl pilocarpic acid esters (107) were found to possess even better ocular delivery characteristics (enhanced absorption and longer lasting pilocarpine activity) than the mono esters (Fig. 7) [328]. Furthermore, the *O*-acylation step gives further possibilities of varying the physicochemical properties of the prodrugs. Some properties of the derivatives are given in Table 20. Finally, it should be added that although the prodrugs are very lipophilic at pH 7 – 7.4 the basic character of the imidazole moiety in the compounds ($pK_a \approx 7.0$) allows the preparation of sufficiently water-soluble salts.

In contrast to pilocarpic acid, the ring-opened hydroxy acid of canrenone is capable of undergoing ready cyclization in neutral aqueous solution [332]. The potassium salt of canrenoic acid (108) is used as a parenteral prodrug form of canrenone (109) due to its better aqueous solubility [332]. In vivo canrenoic acid is

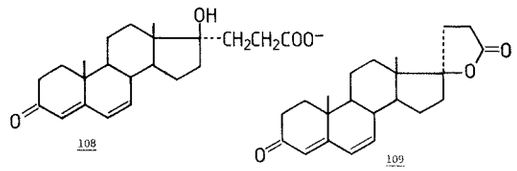
TABLE 20
Rate Data for the Hydrolysis of *O*-Acyl Derivatives of Pilocarpic Acid Esters (107) at 37°C, and Partition Coefficients for the Compounds[a]



R_1	R_2	$t_{½}$ in 75% human plasma (minutes)	k_{OH}[b] (M^{-1} min^{-1})	log P[c]
Benzyl	benzoyl	12	3.8	4.22
4-Chlorobenzyl	benzoyl	17	3.4	4.75
2-Phenylethyl	benzoyl	15	3.8	4.60
4-Methylbenzyl	benzoyl	16	4.0	4.70
4-Methylbenzyl	acetyl	24	12.6	3.16
4-Methylbenzyl	butyryl	5	3.5	4.09
Benzyl	phenylacetyl	4	21.7	3.85
Benzyl	3-chlorobenzoyl	25	14.6	4.93
Benzyl	nicotinoyl	6	44.4	2.90
Benzyl	butyryl	3	3.5	3.63
Benzyl	hexanoyl	4	3.3	4.60

a. From Bundgaard et al. [328, 331].
b. Hydroxide ion catalytic rate constants for the overall hydrolysis of the diesters.
c. Partition coefficients between octanol and 0.05 M phosphate buffer solution of pH 7.40.

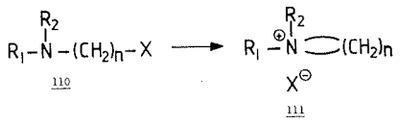
converted rapidly ($t_{½} \approx 8$ minutes) to canrenone, but not quantitatively, since an equilibrium between the two compounds is established [333 – 335]. Only the closed lactone ring form is biologically active [336].



108 109

6.6. Cyclic Quaternary Ammonium Compounds

Due to their high hydrophilic character, quaternary ammonium drugs have poor membrane-penetrating properties, resulting in, e.g., incomplete oral absorption or poor delivery to the brain. This problem may in some cases be overcome by using tertiary *N*-haloalkylamines (110) as prodrugs. Such compounds are able to cyclize to the corresponding quaternary derivatives (111) and are much more lipophilic than these, thus affording improved drug delivery. While Levine et al. [337] pioneered the potential use of this prodrug approach, Ross and co-workers in a series of studies have greatly expanded the concept and applied it to several different agents, such as quaternary analogs of bretylium [338], xylocholine [339], lidocaine [340], troxonium [341] and, recently, the cyclic quaternary ammonium derivatives of the tricyclic psychopharmacological agents, promazine, imipramine and amitriptyline [342]. Most of these studies have been reviewed by Stella [18]. The nucleophilic cyclization reaction occurs readily at physiological conditions of pH and temperature in vivo [343, 344]. The rate of formation of the quaternary product is dependent mainly on the length of the chain connecting the halogen and the nitrogen, the nature of the halogen, and the substituent at the terminal position of the alkyl chain. Bromo derivatives cyclize more rapidly than the corresponding chloro derivatives and, with respect to alkyl chain length, the following order of reactivity is generally observed: butyl > ethyl > pentyl > propyl > hexyl [338 – 342].



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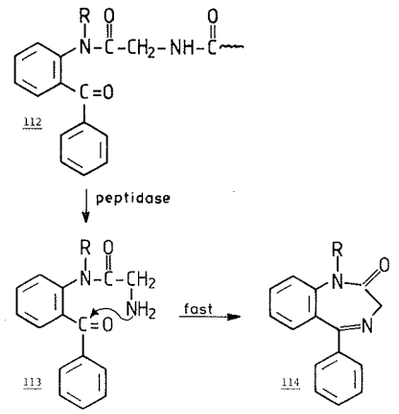
6.7. 1,4-Benzodiazepines

Dipeptide derivatives of 2-aminobenzophenones (112) may be highly water-soluble prodrug forms of diazepam and similar slightly soluble 1,4-benzodiazepinones,

suitable for parenteral administration [345 – 348]. The derivatives are stable in vitro but are cleaved in vivo by peptidases, with formation of a 2-aminoacetamidobenzophenone (113), which subsequently undergoes a spontaneous cyclization to the corresponding benzodiazepine (114) (Scheme 24). The rate of the in vivo hydrolysis of the peptide linkage depends markedly upon the L-amino acid attached to the 2-aminoacetamidobenzophenone, e.g., peptides derived from Phe and Lys are cleaved much faster than those from Gly and Glu [345]. The rate of the spontaneous cyclization of the intermediate (and inactive [349, 350]) 2-aminoacetamidobenzophenone is dependent on the substituents in the phenyl groups as well as on the nitrogen atom. Whereas the benzophenone derived from diazepam cyclizes almost immediately at pH 7.4, that of demethyldiazepam shows a half-time of conversion of 15 minutes at pH 7.4 and 37°C [351]. The cyclization is reversible in weakly acidic solution and the kinetics of this process for various 1,4-benzodiazepines have been described [351 – 357]. Similar ring-opened double prodrug derivatives of triazolobenzodiazepines (e.g., triazolam) have been studied [358 – 360]. The large increase in water-solubility achieved by this prodrug approach can be exemplified with triazolam: its solubility is 0.015 mg ml^{-1} at 25°C whereas the water-solubility of the corresponding glycylaminobenzophenone derivative as hydrochloride salt is 109 mg ml^{-1}, as found by Hirai et al. [358].



Scheme 24

7. Cyclic prodrug derivatives

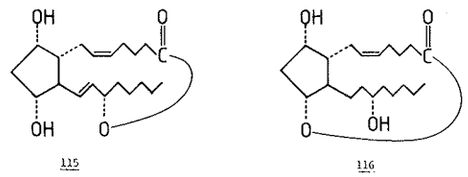
A drug substance can often contain two or more different derivatizable functional groups or two or more of the same functional group. In such cases the formation of cyclic derivatives through bridging these groups may be an interesting and useful chemical approach to obtain prodrug forms, as illustrated in the following.

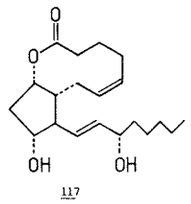
7.1. Lactones

As described in section 6.5, hydroxy acids or esters thereof may be prodrugs for lactone drugs. Conversely, in some cases where the active drug is the open-chain hydroxy acid a suitable prodrug may be the corresponding lactone.

Thus, γ-butyrolactone (104) has been shown to be an excellent prodrug for 4-hydroxybutyric acid (103, R = H) [361 – 364]. The lactone is absorbed faster and to a much greater extent than the hydroxy acid following oral administration, which can be ascribed to its greater lipophilicity as well as to its greater resistance against first-pass metabolism. Besides increasing the bioavailability, the lactone prodrug also confers a more prolonged hypnotic effect for the drug [362, 363]. This effect, which is also seen after intravenous administration, is rather surprising in view of the very rapid hydrolysis of γ-butyrolactone to the parent active hydroxy acid in the blood, by virtue of a γ-lactonase enzyme [365, 366]. A possible explanation is that the lactone has a tissue distribution different from that of the very hydrophilic 4-hydroxybutyric acid and that it may be stored in a tissue depot from which its release is relatively slow [363].

Bundy et al. [367] have recently prepared a number of 1,9-, 1,11- and 1,15-lactones of a variety of biologically interesting prostaglandins and assessed their potential as prodrugs for the corresponding open-chain hydroxy acid parents. In most cases the prostaglandin lactones themselves possessed only very low levels of intrinsic biological activity. In vitro and in vivo studies showed that the 1,15-lactones (115) of prostaglandin (PG) $F_{2\alpha}$ underwent facile enzymatic hydrolysis, while the 1,11-lactones (116) hydrolyzed with intermediate ease and the 1,9-lactones (117) were virtually inert to hydrolysis. Some hydrolysis data for the lactones (115 – 117) of $PGF_{2\alpha}$ are listed in Table 21. While none of the lactone prodrugs afforded significantly prolonged blood levels of the biologically active ring-opened analogues, some PGF lactones including $PGF_{2\alpha}$ 1,15-lactone and (15*S*)-15-methyl-$PGF_{2\alpha}$ 1,15-lactone may represent potentially useful prodrugs for potent fertility-active prostaglandins due to their diminished blood pressure and ger-





bil colon-stimulating activity along with their ease of undergoing enzymatic hydrolysis to the parent prostaglandins [367].

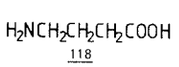
TABLE 21
Hydrolysis of $PGF_{2\alpha}$ Lactones[a]

Compound	% hydrolysis following 20 hours incubation with		
	Saline	Human plasma	Rat plasma
1,15-Lactone (115)	4.4	57.9	58.7
1,11-Lactone (116)	0.2	0.6	80.6
1,9-Lactone (117)	0.2	0.4	0.8

a. From Bundy et al. [367].

7.2. Lactams and pyrrolines

γ-Aminobutyric acid (118) and simple structural analogues such as 4-aminopentanoic acid are not capable of penetrating the blood-brain barrier due to too low lipophilicity of the compounds. Lipophilic, bioreversible ester derivatives may be useful as brain delivery forms of γ-aminobutyric acid and its analogues but, more surprisingly, some cyclic derivatives such as 2-pyrrolidones and Δ′-pyrrolines also appear to represent a chemical class of brain-penetrating prodrug derivatives. Thus, Callery et al. [368] have reported that intravenously administered 2-pyrrolidinone (119) penetrates readily into the CNS of mice and is converted enzymatically to γ-aminobutyric acid in the brain. In another study, Callery et al. [369] showed that Δ′-pyrroline (120) and its analogues can serve as lipophilic prodrugs for γ-aminobutyric acid and its corresponding analogues. Liver and brain homogenates from mice were found to contain enzymes capable of oxidizing the pyrrolines to the parent amino acids and, following intraperitoneal injection of the prodrugs to mice, substantial amounts of the parent amino acids were detected in the brain.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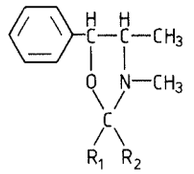
7.3. Oxazolidines as prodrugs for β-aminoalcohols

There are several drugs containing a β-aminoalcohol moiety (e.g., various sympathomimetic amines and β-blockers) which may exhibit delivery problems, e.g., due to unfavourable solubility or lipophilicity characteristics as well as pronounced first-pass metabolism. Oxazolidines have recently been proposed [287–289] as

potentially useful prodrug candidates for the β-aminoalcohol function (see Scheme 21).

Oxazolidines are cyclic condensation products of β-aminoalcohols and aldehydes or ketones and they undergo a facile and complete hydrolysis in aqueous solution. By varying the carbonyl moiety it is possible to control the rate of formation of a given β-aminoalcohol. Thus, the following half-lives of hydrolysis for various (−)-ephedrine oxazolidines (121) were found at pH 7.4 and 37°C [288, 289]: 5 minutes (benzaldehyde), 5 seconds (salicylaldehyde), 4 seconds (formaldehyde), 17 seconds (propionaldehyde), 30 minutes (pivaldehyde), 4 minutes (acetone) and 6 minutes (cyclohexanone). The hydrolysis rates at neutral and basic pH decrease with increasing steric effects of the substituents derived from the carbonyl component as well as with increasing basicity of the oxazolidines [288]. Structure-reactivity relationships have also been established for the influence of the β-aminoalcohol moiety upon the stability [287]. It was found (cf. Table 15) that the hydrolysis rates at neutral and basic pH decreased with increasing steric effects within this moiety, in particular at the α-position to the nitrogen atom, and increased with increasing electron-negativity of the substituents at the β-position to the nitrogen atom [287]. Obviously, such relationships may be useful for the prediction of the reactivity of an oxazolidine to be designed as a prodrug derivative of a drug molecule containing a β-aminoalcohol moiety (or a carbonyl group).


121

It may be of interest to note that the cyclic oxazolidine-related derivatives obtained from γ-aminoalcohols and ketones behave hydrolytically as the corresponding oxazolidines [287].

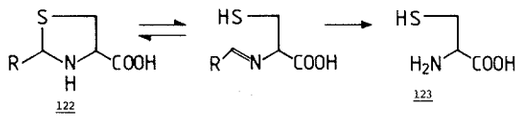
Oxazolidines are much weaker bases (pK_a 6–7) than the parent β-aminoalcohols, and therefore they are more lipophilic than these at physiological pH [288]. Such increased lipophilicity may become advantageous in situations where delivery problems for β-aminoalcohol-type drugs are due to low lipophilicity, e.g., in case of dermal absorption. A drawback of oxazolidines as prodrugs is their poor stability in aqueous solution, raising formulation-stability problems, at least for solution products. For most oxazolidines, however, the rates of decomposition are greatest at neutral and basic pH and increased stability is attained at acidic non-physiological pH values [287–289]. A really effective solution of the in vitro stability problem would be further derivatization of the oxazolidines to produce compounds whose initial cleavage to oxazolidine relies on enzymatic catalysis. Studies (unpublished) in this laboratory have shown that *N*-acetylated or *N*-benzoylated oxazolidines

derived from primary aminoalcohols are in fact highly stable in aqueous solution but, unfortunately, they are also resistant to hydrolysis by plasma enzymes. Attempts to find more promising kinds of *N*-acylation or other means of derivatization of the oxazolidine nitrogen function are currently being made.

A description of oxazolidines as prodrug forms for drugs containing aldehydic or ketonic groups is given in section 5.2.

7.4. Thiazolidines as prodrugs of cysteine

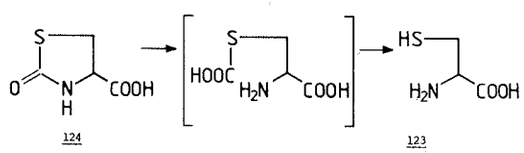
Various 2-substituted thiazolidine-4(*R*)-carboxylic acids (122) have been proposed as prodrugs of L-cysteine (123) and evaluated for their protective effect against hepototoxic deaths produced in mice by paracetamol [370, 371]. It was shown that such compounds may be useful prodrugs, possessing less toxicity and greater efficacy than cysteine and capable of liberating this sulphydryl amino acid in vivo intracellularly by nonenzymatic ring opening, with the intermediate formation of an unstable Schiff base (Scheme 25). Once formed in the liver, L-cysteine stimulates the biosynthesis of glutathione, which is a required cofactor for the glutathione *S*-transferases of liver that conjugate and detoxify the reactive metabolites generated in the biotransformation of paracetamol or other xenobiotics. Administration of cysteine per se is not an ideal way to increase glutathione concentrations because the amino acid is metabolized rapidly and is somewhat toxic, partly due to its extracellular effects [372].



122 123

Scheme 25

Other studies [372 – 374] have indicated the potential usefulness of the related 2-oxothiazolidine-4(*R*)-carboxylic acid (124) as a prodrug form for the intracellular delivery of L-cysteine. 2-Oxothiazolidine-4(*R*)-carboxylic acid is a good substrate of the intracellular enzyme, 5-oxoprolinase [373], which opens the thiazolidine ring to yield *S*-carboxy-L-cysteine, which in turn undergoes a spontaneous decarboxylation to give L-cysteine (Scheme 26).



124 123

Scheme 26

The use of thiazolidines as prodrugs of aldehydic or ketonic drugs is described in section 5.2.

7.5. 4-Imidazolidinones as prodrugs for the α-aminoamide moiety

In recent years several biologically active peptides have been discovered, including peptides consisting of only two amino acids; however, the clinical application of such compounds is seriously hampered due to substantial delivery problems [375 – 377]. Peptides are poorly absorbed following oral administration and they suffer from metabolic lability from hydrolysis by plasma and tissue peptidases, making even simple parenteric administration problematic. Several peptides also suffer from systemic transport problems in that they do not readily penetrate cell membranes to reach the receptor biophase or cross the blood-brain barrier. Derivatization of bioactive peptides to produce prodrugs may possibly overcome some of these delivery problems.

The α-aminoamide moiety is found in almost all peptides, and a potentially useful and broadly applicable prodrug type for this group may be 4-imidazolidinones (Scheme 27), as recently suggested by Klixbüll and Bundgaard [378]. These authors studied the hydrolysis kinetics of five 4-imidazolidinones derived from acetone and the dipeptides Ala-Gly, Ala-Ala, Phe-Leu, Leu-Gly and Asp-Phe methyl ester (Fig. 8). The imidazolidinyl peptides, which may be regarded as cyclic *N*-Mannich bases, were shown to undergo a complete hydrolysis in the pH range 1 – 10 at 37°C and most of them showed a sigmoidal pH-rate profile, with maximum rates at pH >

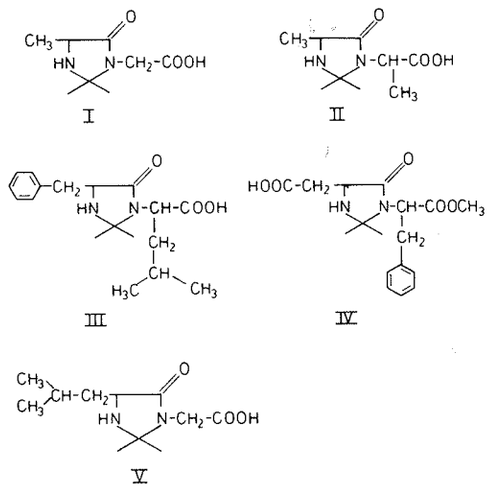
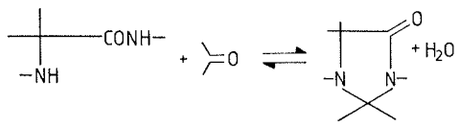


Fig. 8. Chemical structures of various 4-imidazolidinones investigated by Klixbüll and Bundgaard [378], with the parent dipeptides (all of L-configuration) given in parentheses: I (Ala-Gly), II (Ala-Ala), III (Phe-Leu), IV (Asp-Phe methyl ester) and V (Leu-Gly).

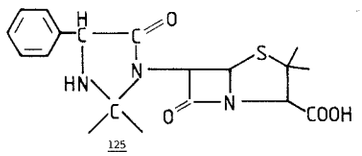


Scheme 27

4. The stability of the derivatives varied widely, the following half-lives being obtained at pH 7.40 and 37°C: 0.8 hours (Asp-Phe methyl ester), 3.4 hours (Phe-Leu), 24.6 hours (Ala-Ala), 410 hours (Ala-Gly) and 530 hours (Leu-Gly). These rates might not be expected to change much in vivo [378]. The major structural factor influencing the stability appears to be the steric properties within the C-terminal amino acid residue. However, for the further evaluation of 4-imidazolidinones as a prodrug type for peptides it is of importance to establish the effect of the carbonyl component on the reactivity of the derivatives. In the case of 4-imidazolidinones derived from ampicillin, which may be regarded as a model of a peptide containing an α-aminoamide moiety, the rate of 4-imidazolidinone hydrolysis shows only a small dependence on the carbonyl component (aldehyde or ketone) [379], but this does not necessarily apply to other imidazolidinyl peptides.

In considering 4-imidazolidinones as a potential prodrug type for peptides the large decrease obtained in basicity of the reacting N-terminal amino group should be appreciated. The 4-imidazolidinones mentioned above are much weaker bases (pK_a about 3.1) than the parent dipeptides and such depression of amino protonation brings about an increase in the lipophilicity of the N-terminal amino acid part at physiological pH, as confirmed by partition experiments in octanol-aqueous buffer systems [378]. The increased lipophilicity attained, which obviously will be influenced further by the lipophilicity of the substituents of the carbonyl component, may be of value in situations where delivery problems of peptide drugs are due to low lipophilicity.

There is already one 4-imidazolidinone prodrug derivative in clinical use, namely hetacillin (125), formed by condensation of ampicillin with acetone [380]. It is readily hydrolyzed to the active ampicillin in aqueous solution, the half-life being 15 – 20 minutes at pH 4 – 8 and 35°C [381] and about 11 minutes in vivo, as determined after intravenous administration in man [382, 383]. An advantage of hetacillin is its higher stability in concentrated aqueous solutions [384] compared with ampicillin sodium, which undergoes a facile intermolecular aminolysis by attack of the side-



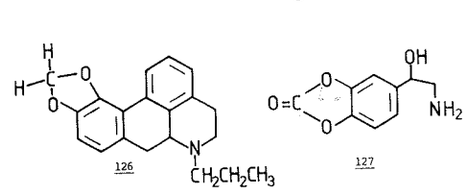
125

chain amino group in one molecule on the β-lactam moiety of a second molecule [385].

7.6. Cyclic prodrugs for the catechol group

The clinical usefulness of apomorphine and *N-n*-propylnorapomorphine as dopamine-receptor agonists is limited by poor oral bioavailability and short duration of action. Although labile lipophilic diesters can serve as prodrugs of apomorphine, resulting in an increased duration of action, they are not active orally, primarily due to rapid hydrolysis in the intestine [386]. Better prodrug candidates for the metabolically vulnerable catechol system appear to be cyclic ethers. Various substituted and unsubstituted 10,11-methylenedioxy derivatives of apomorphine and *N-n*-propylnorapomorphine have been prepared, and one of these, 10,11-methylenedioxy-*N-n*-propylnorapomorphine (126), was found to be both a long-acting and an orally effective prodrug [387 – 391]. While the oral activity of this prodrug certainly reflects protection of the catechol system of the parent drug from first-pass metabolism, the conversion of the prodrug depends on the action of hepatic microsomal enzymes [391].

Another means of cross-linking the catecholic hydroxyl groups is by cyclic carbonate ester formation. Such a derivative (127) of L-norepinephrine has been described [392] as a bioreversible lipophilic prodrug form capable of crossing the blood-brain barrier to a greater extent than the parent norepinephrine.



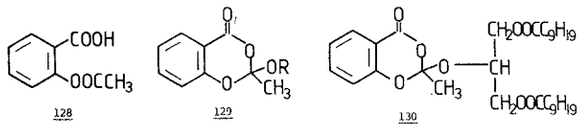
126 127

7.7. Cyclic derivatives of acetylsalicylic acid

With the exception of some special esters [44, 45] and acylal [393, 394] derivatives most of the considerable number of 'aspirin prodrugs' which have been described in the literature are essentially salicylic acid prodrugs since they release salicylic acid and not acetylsalicylic acid (128) upon hydrolysis. Recently, Hansen and Senning [395] have shown that by incorporating both the carboxyl and the acetoxy ester group into an *ortho* ester function it may be feasible to obtain true aspirin prodrugs. A number of 2-substituted 2-methyl-4H-1,3-benzodioxin-4-one derivatives (129) was prepared and investigated in terms of in vitro non-enzymatic hydrolysis at neutral pH. Of the compounds studied, only the 2-*tert*-butoxy derivative was observed to be hydrolyzed to acetylsalicylic acid, the half-life being a few minutes at pH 7.4 and 37°C. It was concluded that a tertiary alkoxy group at the C-2 posi-

tion is necessary in order to give acetylsalicyclic acid and not salicylic acid as the major product of hydrolysis [395]. However, to assess more fully the potential usefulness of such cyclic derivatives as prodrugs information on the hydrolysis in the presence of enzymes from, e.g., plasma is certainly needed.

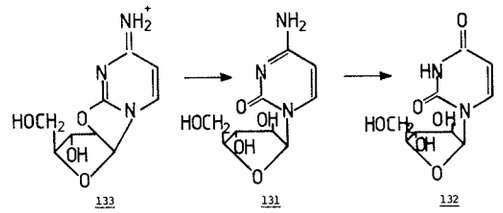
A glyceride derivative (130) of a similar structure has been obtained by Paris et al. [47]. It was characterized in whole animal studies as being roughly equipotent to acetylsalicylic acid on a molar basis, while being essentially devoid of ulcerogenic properties. However, whether the derivative is cleaved to acetylsalicylic acid and not to salicylic acid has not been determined.



128 129 130

7.8. Cyclic cytarabine prodrug

Cytarabine (131) is an effective antileukaemic agent, but it has a very short half-life in vivo because of its rapid deamination by cytidine deaminase to give the inactive 1-β-D-arabinosyluracil (132). The cyclic compound ancitabine (133) has shown promise as a prodrug with improved pharmacokinetic properties. It is resistant to cytidine deaminase and has been found to extend the biological duration of cytarabine due to slow hydrolytic conversion to the drug in vivo [396, 397]. The hydrolysis is chemically, not enzymatically, mediated and it proceeds by hydroxide ion- and water-catalyzed reactions [398].



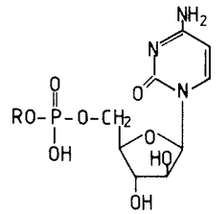
133 131 132

8. Prodrugs for the phosphate group

A vital step in the mode of action of purine and pyrimidine nucleosides against viral and neoplastic diseases is their conversion into their 5′-mono-, di- or triphosphates by cellular or virus-induced kinases [399, 400]. The nucleoside form is most often administered because of the ease with which it penetrates cells but, unfortunately, several nucleoside derivatives fail to undergo the necessary phosphorylation to the

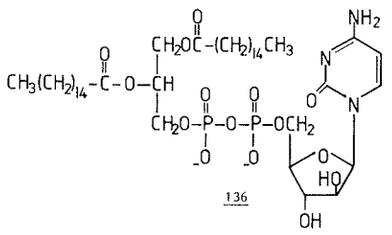
active nucleotide form (development of resistance) [400]. On the other hand, the nucleotides per se are not directly applicable as chemotherapeutic agents because of their poor penetration of cell membranes and rapid dephosphorylation to the parent nucleosides by nonspecific serum phosphohydrolases, such as alkaline and acid phoshatases [401, 402]. Since masking of the ionized and hydrophilic phosphate group might be expected to increase the penetrability of the nucleoside phosphates, the development of suitable biologically reversible phosphate protective groups constitutes an important task.

Various potentially useful prodrug derivatives for the phosphate group of nucleotides have been exploited. For example, the ionic phosphate moiety can be converted to an ester function to obtain less ionic character and better cellular penetrability. Upon entry into cells, enzymatic hydrolysis would be expected to occur. Revankar et al. [403] have described the synthesis and properties of the monomethyl phosphate ester of Ara-A (vidarabine) and Rosowsky et al. [404] have evaluated a series of lipophilic 5′-alkyl phosphate esters of Ara-C, N^4-acyl Ara-C derivatives and anhydro-Ara-C. Of the simple 5′-alkyl phosphate esters of Ara-C 5′-monophosphate (134) tested in culture against L 1210 leukaemic cells, only the 5′-glyceryl phosphate ester (135) showed activity comparable to the parent nucleotide. The observed lack of in vivo activity of the 5′-butyl phosphate ester and related alkyl phosphate esters was ascribed to rapid degradation of the esters in the host circulation so that the amount of intact phosphodiester reaching the tumour was too small to be biologically significant [404]. More successful prodrug derivatives of Ara-C appear to be some phospholipid compounds. Matsushita et al. [405] have shown that the 5′-diphosphate-L-1,2-dipalmitin derivative of Ara-C (136) exhibits a much increased efficacy relative to the parent drug against L 1210 lymphoid leukaemia in mice. Upon cellular uptake, the derivative is enzymatically cleaved, leading to the release of Ara-C 5′-monophosphate. It is also noteworthy that the by-product (i.e., L-α-phosphatidic acid) from this intracellular hydrolysis is a natural component of cell membranes. The aggregational and morphological characteristics of structure (136) and related liponucleotides have been studied and discussed in relation to the biological efficacy of these prodrugs [406]. Cyclic 3′,5′-phosphates of Ara-A [403] and Ara-C (137) [407] have been prepared. Such



134 R = H

135 R = $CH_2OH-CHOH-CH_2-$



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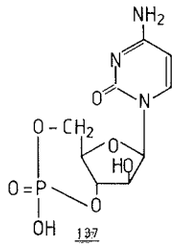

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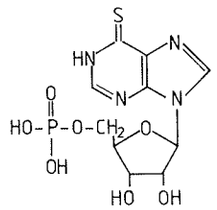

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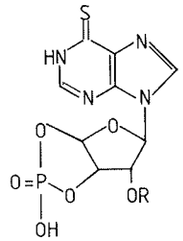
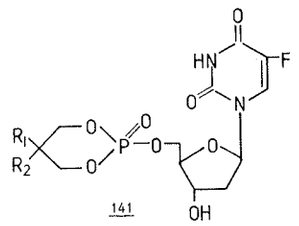


139 R = H

140 R = acyl

internal ester derivatives are more lipophilic than the parent monophosphate nucleosides and may be converted to these intracellularly by phosphodiesterases [408]. Intramolecular phosphate ester formation has also been explored for thioinosinic acid (138) [409] in an attempt to obtain lipophilic and readily membrane-penetrating prodrugs. Thioinosinic acid is formed as a first step in the cytotoxic action of 6-mercaptopurine, but due to its ionic character it does not penetrate cell membranes. Removal of one negative charge from thioinosinic acid by formation of the cyclic 3′,5′-phosphate ester (139), however, has only a minimal effect on cell penetration, but further derivatization of this compound by 2′-*O*-acylation (140) with, e.g., palmitic acid greatly improved the lipophilicity, resulting in enhanced cytotoxicity in vitro [409]. These acylated cyclic nucleotides (140) were shown to enter readily S49 mouse lymphoma cells and then be converted, by phosphodiesterase action and deacylation, to the parent thioinosinic acid [409].

An illustration of the different chemical approaches being developed for the bioreversible derivatization of the phosphate groups of nucleotides in order to in-



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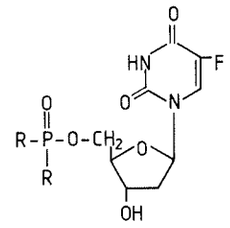


142 R=OH

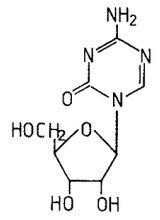
143 R=e.g. NH_2

crease their lipophilicity and membrane permeability is provided by studies concerned with 2′-deoxy-5-fluorouridine. Hunston et al. [410] recently have prepared a series of cyclic phosphotriester derivatives of this nucleoside (141). Such derivatives are uncharged, and consequently are much more lipophilic than the parent nucleoside 5′-phosphate (142). A large difference in cytotoxicity between the derivatives tested was observed, which was ascribed to their varying stability in aqueous solution [410]. The possible susceptibility of the phosphotriesters to enzymatic hydrolysis was not examined. Other neutral and lipophilic derivatives of 2′-deoxy-5-fluorouridine which have been investigated are phosphorodiamidates (143) [411], i.e., esters of phosphorodiamidic acid. Phosphoroamidates and phosphorodiamidates are relatively labile in aqueous solution and are hydrolyzed to the parent phosphates at rates that are influenced by pH of the medium and the nature of the amino group (steric and polar effects) [412]. Besides, the *O*-substituent, if being a nucleoside, may have a pronounced influence upon the rate of hydrolysis through conformational effects [411]. For the 5′-phosphorodiamidate derivatives (143), their cytotoxic activity as assessed in culture was found to increase with increasing hydrolytic lability in acidic aqueous solution. Also, for this type of phosphate derivatives the possibility of enzymatic hydrolysis has not been studied.

O-Imino esters of *N,N*-bis(2-chloroethyl)phosphorodiamidic acid have been evaluated as prodrugs [412a]. The derivatives proved stable toward hydrolysis at neutral pH but are capable, albeit slowly, of undergoing enzymatic reduction of the N-O bond by liver enzymes, to release the parent phosphoramide mustard.

A newly described and potentially useful phosphate prodrug type is *O*-

resulted in a derivative (149) which possesses increased stability in acidic solutions but, on the other hand, decomposes quickly at neutral (physiological) pH to give back the parent drug. Not only is this azacytidine prodrug suitable as a delivery form for prolonged infusions but due to its stability at acidic pH it also holds promise as a means of improving the oral absorption of the acid-labile azacytidine [422].



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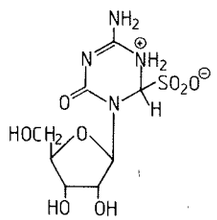

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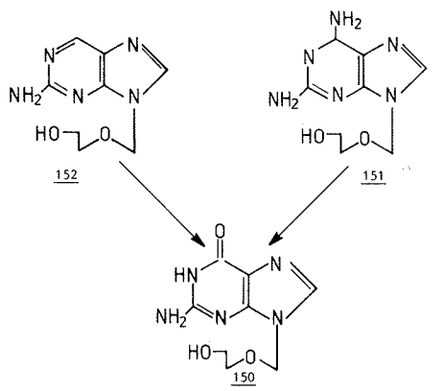
9.3. Enzyme-specified prodrugs of acyclovir

Acyclovir (150) is a clinically useful antiherpetic agent which exhibits great selectivity in its antiviral action through conversion to the active phosphorylated species by virtue of virus-specific thymidine kinase [423 – 425]. It suffers, however, from poor oral bioavailability, only 10 – 20% of an oral dose being absorbed in humans [426 – 429]. This can most probably be ascribed to the poor water-solubility and lipophilicity of the compound. The 6-deoxy-6-amino congener (151) of acyclovir has been studied as a prodrug in an attempt to improve the oral bioavailability [430]. It is deaminated to acyclovir by adenosine deaminase [431], but oral dosing of dogs and rats with the prodrug resulted in only modest increases in acyclovir plasma levels relative to those achieved with acyclovir itself [430]. A far better prodrug may be 6-deoxyacyclovir (152), recently developed by Krenitsky et al. [432]. This compound is 18 times more water-soluble than acyclovir and is oxidized rapidly in vivo

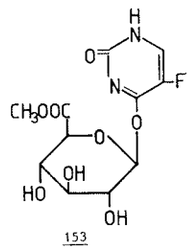


152 151 150

by xanthine oxidase to the parent drug. Preliminary studies in rats and in human volunteers showed that 6-deoxyacyclovir is absorbed readily after oral administration (5 – 6 times greater bioavailability relative to acyclovir) [432, 432a]. The compound is also susceptible to oxidation by aldehyde oxidase, to give the inactive 8-hydroxy-6-deoxyacyclovir, but this non-activating oxidation apparently plays only a minor role in comparison to the activating oxidation by xanthine oxidase [432].

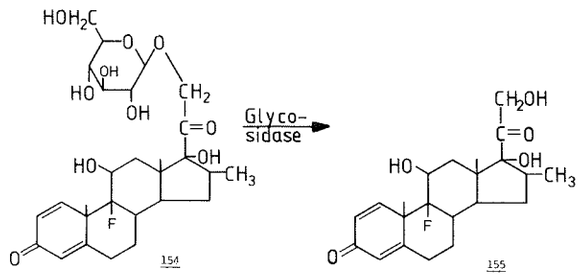
9.4. Glycosidic prodrugs

Site-specific drug delivery through a site-specific prodrug activation may be accomplished by the utilization of some specific property at the target site for the prodrug→drug conversion. Glycosidases exist in higher amounts in certain tumour tissues than in normal cells and, therefore, the concept of using glucuronides or glycosides as prodrug forms for anticancer agents offers promise as a means to improve the therapeutic value of such drugs by affording a target-specific release [23]. Studies along this direction are under way in several laboratories. For example, Arakawa et al. [433] have reported that the glycosidic 5-fluorouracil derivative (153) shows a higher degree of tumour-specific activity in mice with transplantable solid tumours than the parent drug. Likewise, a glucuronide of 6-mercaptopurine was shown to be a good substrate of β-glucuronidase and to possess a selective activity against L 1210 cells via preferential bioactivation in tumour cells [434].



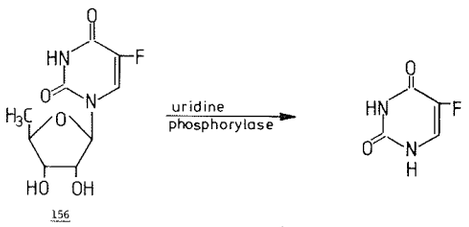
153

The unique glycosidase activity of the colonic microflora has been utilized recently as the basis for obtaining a colon-specific drug delivery [435]. Glycoside derivatives are hydrophilic and, thus, are poorly absorbed from the small intestine. Once such a glucoside reaches the colon it can be cleaved by bacterial glycosidases, releasing the free drug to act on the colon or be absorbed by the colonic mucosa. This concept was tested in rats with two steroid prodrugs, dexamethasone 21-β-D-glucoside (154) and prednisolone 21-β-D-glucoside [435], two prodrugs that may be useful in treating inflammatory bowel disease. Of these glycosidic prodrugs tested, the dexamethasone glucoside appeared to be a good candidate as nearly 60% of an oral dosis of the prodrug reached the caecum in the form of free steroid (155). When the parent steroids were administered orally, they were absorbed almost exclusively from the small intestine; less than 1% reached the caecum.



9.5. Miscellaneous

5′-Deoxy-5-fluorouridine (156) is a recently developed prodrug of 5-fluorouracil that shows high antitumour activity against experimental tumours in animals and causes considerably less host toxicity compared to 5-fluorouracil [436 – 442a]. There is strong evidence that the activity of 5′-deoxy-5-fluorouridine and its favourable therapeutic index are due to preferential bioactivation in tumour cells to the active 5-fluorouracil by uridine phosphorylase (Scheme 29). The activity of this enzyme has been found to be markedly higher in various tumour tissues than in the surrounding normal tissues [438, 441].



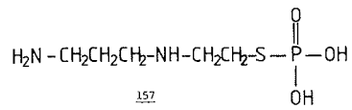
Scheme 29

N- and *O*-Triorganosilylation has been proposed as a potentially useful prodrug approach to, e.g., increase the lipophilicity. Beckett et al. [443] have studied the hydrolysis and partitioning behaviour of various *O*-trialkylsilyl derivatives of ephedrine and related aminoalcohols and Chiu et al. [444] recently described various *N*- and *O*-triorganosilylated derivatives of cyclophosphamide, nitrogen mustards, 5-fluorouracil and other anticancer agents. The hydrolysis kinetics was examined and it was concluded that triorganosilyl groups can be used as hydrolytically labile moieties for the design of anticancer prodrugs.

N-Halogenated derivatives may be prodrugs of phosphoramides, sulphonamides or carboxamides, as suggested by Zon et al. [445]. These authors showed that 3-halocyclophosphamides are reduced to cyclophosphamide by liver enzymes.

Due to their relative stability in vivo, simple amides have been used only to a

limited extent as prodrugs for carboxylic acids (see also section 4.1); esters are most often preferred. Examples of amide prodrugs for drugs containing a carboxyl group include various amides of methotrexate [446, 447].



Thiols may be derivatized by ester formation. A phosphate ester of 2-mercaptoethyl-1,3-diaminopropane (157) has been shown to be a useful prodrug, improving the oral absorption of the parent mucolytic agent [448]. Another way of derivatizing thiols is by making disulphides which are reducible in vivo. A number of disulphide prodrugs of antimicrobially active thiols have recently been described in a patent [449].

Finally, the aspect of using macromolecules as the pro-moiety in prodrugs should be mentioned. This is an area in which there is great activity at present, and several carriers are considered, such as proteins, polypeptides, polysaccharides, nucleic acids, monoclonal antibodies, etc. [450 – 453]. In most cases such drug-carrier conjugates are only active after cleavage with release of the parent drug, and thus they are to be considered as prodrugs. Many of the chemical approaches used in the preparation of low-molecular-weight prodrugs may also be applied for the development of macromolecular prodrugs, especially if the possibility of using spacer groups for linking the drug to the carrier is considered. Thus, mitomycin C has been linked to dextran through the use of 6-aminohexanoic acid as a spacer [454 – 457] and daunorubicin to albumin through an oligopeptidic spacer [458].

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Design of Prodrugs (Bundgaard, H., ed.)

CHAPTER 2

Design of prodrugs based on enzyme-substrate specificity

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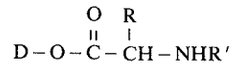
1. Introduction

A number of inherent undesirable properties may preclude the use of a drug molecule in clinical practice, and drug derivatization has been long recognized as an important means of producing more efficacious pharmaceutical products. Central to the prodrug design is in vivo reconversion. The drug derivative must be reconverted rapidly, or at a controlled rate, to the active therapeutic agent in vivo while at the same time be sufficiently stable in vitro such that a stable pharmaceutical product can be developed. In the rational design of prodrugs, it is necessary to consider (a) what structural modifications of the parent molecule are necessary to reduce or eliminate the particular undesirable effect, and (b) what conditions are available in vivo (enzymes, pH, etc.) to regenerate the parent molecule from the prodrug. For example, design of prodrugs through consideration of enzyme-substrate specificities was suggested [1] and, based on known substrate specificities for intestinal enzymes, a rationale was established for designing amino acid derivatives of aspirin [2]. In vitro enzyme kinetics of reconversion have also been reported [3, 4].

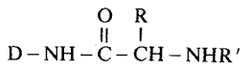
The effective design of prodrugs through the consideration of enzyme-substrate specificities requires considerable knowledge of the particular enzyme or enzyme system. In addition to the enzyme's specificity (both kinetic and binding), the type of reaction catalyzed, the enzyme distribution and level, and the functional role of the enzyme in the cellular biochemistry should be known. Given that a drug has a free carboxyl, amino or hydroxyl group, corresponding esters or amides of amino acids can be made so as to alter the physical properties in almost any desired direction from that of the parent drug, with one or more of the hydrolase enzymes serving as the in vivo reconversion site(s).

TABLE 1
Possible Changes in Physical Properties of Drugs with Free OH Groups



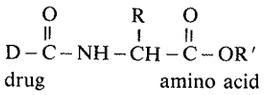
Drug	Amino acid	R′	Change in physical properties
Nonpolar	Polar-acidic-basic	H, $COCH_3$	More polar-ionic
Polar	Nonpolar	$COCH_3$	Less polar
Liquid at room temperature	Polar-nonpolar	H	To a salt
Low aqueous solubility	Acidic-basic	$HCOCH_3$	To acid or base (salt)

TABLE 2
Possible Changes in Physical Properties of Drugs with Free NH_2 Groups



Drug	Amino acid	R′	Change in physical properties
Weak base	Nonpolar	$COCH_3$	To a neutral compound
Weak base	Acidic	$COCH_3$	To an acidic compound
Zwitterionic	Polar-nonpolar	$COCH_3$	To an ionic (acidic) compound
Weak base	Polar-nonpolar	H	Change in pK_a

TABLE 3
Possible Changes in Physical Properties of Drugs with Free COOH Groups



Drug	Amino acid	R′	Change in physical properties
Weak acid	Polar-nonpolar	H	Change in pK_a
Weak acid	Basic	H	Zwitterionic compound
Weak acid	Basic	OC_2H_5	To a basic compound
Zwitterionic	Polar/nonpolar	OC_2H_5	To a basic compound

The diverse properties of the various amino acid residues, combined with the fact that usually they are nontoxic, gives this approach wide applicability. Classifying the amino acids as nonpolar, polar, acidic and basic, a given drug molecule may be made more or less polar, or more or less soluble in a given solvent. Its acid/base properties may be altered or changed completely (i.e., converted from acidic to basic drug or vice versa), or it may be converted from an ionic to a neutral compound, or from neutral to an ionic compound (acidic or basic). Tables 1 – 3 briefly summarize some of these possibilities.

Delivery problems that suggest a drug-chemical modification for oral administration include drug-induced damage to the gastrointestinal tissue, poor aqueous stability [4], extensive first-pass metabolism [5], poor aqueous solubility [6], limited water to membrane partition coefficient [7] and a high degree of ionization over the gastrointestinal pH range [8]. The first three problems are usually the result of a particular functional group which can be derivatized to alter the undesirable physicochemical properties of the molecule.

Commonly cited prodrug forms are simple organic esters or amides of the parent drug. Such prodrugs can be targeted for regions close to absorption sites or sites of action where physiologic conditions are such that nonenzymatic prodrug hydrolysis is a favorable situation. In addition, these prodrugs can be targeted for regions containing high levels of nonspecific esterases. Such strategies have been utilized for drug transport in ophthalmic tissue (epinephrine delivery for glaucoma) [9], skin (vidarabine use as an antiviral agent) [10], and to overcome problems for gastrointestinal absorption (ampicillin [11], carbenicillin [12], and aspirin [13, 14]). The derivatizing group can be selected to increase or decrease drug polarity, thus modifying aqueous or membrane transport properties as well as masking or protecting problematic functional groups. The most significant limitation on use of general ester prodrugs is the lack of specificity with respect to their sites of reconversion.

The body's handling of nutritional substances suggests that the use of a nutrient moiety as a derivatizing group to modify drug physicochemical properties which limit gastrointestinal drug absorption might also permit more specific targeting for enzymes involved in the terminal phases of digestion. Such enzymes include those responsible for the digestion of carbohydrates, fats, protein, and mineral-containing nutrients and the intestinal transport of sugars, lipids and fatty acids, peptides and amino acids, as well as various electrolyte species. These prodrugs have the additional advantage of producing nontoxic nutrient by-products upon cleavage. For example, prodrugs of aspirin have been made using a 2-deoxyglucose moiety [15] to modify the drug's gastric irritation problems. However, this prodrug was not targeted for specific enzymes but was designed to cleave rapidly in solution over a pH range of 3 – 9. Fatty acid esters of acetaminophen have been suggested as prodrugs when coadministered with pancreatic lipase and calcium. Variation in chain length of the fatty acid derivatizing groups was shown to control the rate of

hydrolysis, which had resultant impact on the duration of action of the parent drug [16]. Amino acid esters of drugs have been suggested as prodrugs for the purpose of modifying drug solubility, dissolution rate, gastric irritation, and chemical stability [17, 18].

2. Enzyme classes

The body's handling of nutritional substances suggests that the use of a nutrient moiety as a derivatizing group to modify drug physicochemical properties permits more specific targeting for enzymes. Enzymes are classified according to function into six major groups, and then into several subgroups specifying precisely the actual reaction catalyzed [19]. The six main classes are: 1, oxidoreductases – which catalyze oxidation-reduction reactions; 2, transferases – which transfer groups from one acceptor to another; 3, hydrolases – which catalyze hydrolytic reactions; 4, lyases – which remove groups from substrates by means other than hydrolysis, leaving double bonds; 5, isomerases – which catalyze isomerization reactions; and 6, ligases – which catalyze the joining of two molecules in a reaction coupled with the hydrolyses of a pyrophosphate bond in ATP.

Since this report focuses on enzymes which may be generally useful sites for prodrug reconversion, the specific enzymes reviewed are taken from the hydrolase class.

3. Enzyme kinetic considerations

The general form of the Henri-Michaelis-Menten equation [20] is

$$v = \frac{V_{max}}{1 + K_m/[s]}$$

where v is the velocity at a given substrate concentration, [s], and V_{max} and K_m are complex kinetic constants which depend on the reaction mechanism, pH, temperature, and concentration of other species such as metal ions, inhibitors, etc.

We can illustrate the rate enhancements effected by enzymes by considering the serine proteinases which appear to function according to the following stepwise kinetic scheme [21]:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \underset{P_1}{EP_2} \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E + P_2 \qquad (1)$$

Assuming steady-state conditions and $E_o \ll [s]$, the following equation can be obtained:

$$v = \frac{k_{cat} E_o}{1 + K_m/[s]} \qquad (2)$$

where,

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3}$$

$$K_m = \frac{k_3}{k_2 + k_3} K_s$$

$$K_s = \frac{k_1}{k_{-1}}$$

E_o = enzyme concentration. Under saturation conditions (i.e., when $[s] \gg K_m$)

$$v = V_{max} = k_{cat} E_o$$

Hence, k_{cat}, often referred to as the turnover number, is the maximal number of moles of substrate hydrolyzed per mole of enzyme per unit time. A very rough

TABLE 4
Estimated Range of In Vivo Reconversion Rates

Maximal rate (zero order)	Minimum rate (first-order)[a]
$v = k_{cat} E_O$	$v = \frac{K_{cat} E_o S}{K_m}$
$= (100)(10^{-6})$	$= \frac{10^2}{1} 10^{-6}$ [S]
$= 10^{-4}$ M/second	$= 10^{-4}$ [S]
$k_0 = 10^{-4}$ M/second	$k_1 = 10^{-4}$ sec^{-1}
$t_{1/2} = 5 \times 10^{-5}$ second ($S_o = 10^{-8}$ M)	$t_{1/2} = 6.93 \times 10^3$ seconds
$= 5$ seconds ($S_o = 10^{-3}$ M)	≈ 2 hours

$E_o = 10^{-6}$ M, $k_{cat} = 100$ sec^{-1}.
a. Assuming $K_m = 1$ M.

estimate of possible reconversion rates can be made as follows. A reasonable range for enzyme concentrations in vivo is 10^{-4} M – 10^{-8} M [22]. A reasonable dose range is $10^{-2} - 10^{-5}$ moles of drug and a reasonable serum or tissue concentration is $10^{-3} - 10^{-8}$ M. Table 4 (Ref. 1) presents the estimated range of reconversion rates for the two cases, (i) [s] >> K_m and (ii) K_m >> [s]. From the table it is clear that very rapid reconversion rates are possible. These comparisons emphasize the obvious interest in designing prodrugs with rapid enzymatic reconversion rates. Drug derivatives can be prepared which exhibit long shelf lives in normal dosage forms, but are reconverted rapidly to the active drug in vivo. The reciprocal of Eqn. 2, also called the Lineweaver-Burk equation

$$\frac{1}{V_o} = \frac{1}{k_{cat}E_o} + \frac{K_m}{k_{cat}E_o}\ \frac{1}{S_o} \tag{3}$$

provides a convenient method of analyzing the enzyme kinetic data. If the enzymatic reaction follows Michaelis-Menten kinetics a plot of $1/v_o$ vs. $1/S_o$ provides a straight line with slope $(K_m/k_{cat}E_o)$ and intercept $(1/k_{cat}E_o)$.

An example of such a plot is shown in Figure 1 for α-chymotrypsin-catalyzed hydrolysis of aspirin phenyllactic ethyl esters. It has been shown [23] that k_{cat}/K_m

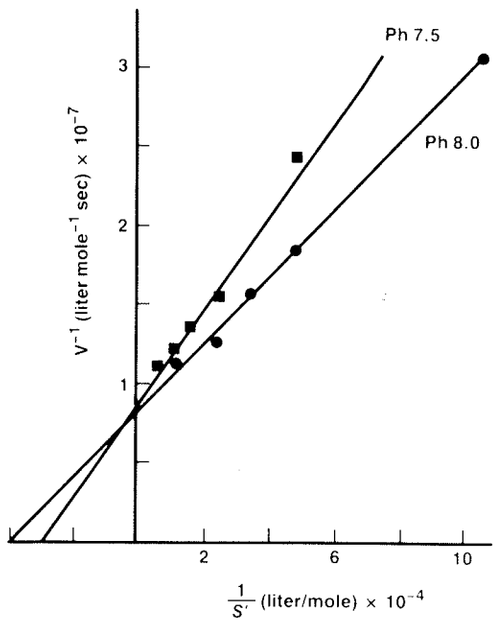


Fig. 1. Lineweaver-Burk plot for the hydrolysis of III by 3.02×10^{-5} M α-chymotrypsin at pH 7.5 and by 1.5×10^{-5} M α-chymotrypsin at pH 8.0.

is the most meaningful kinetic parameter for comparing different substrates. Good substrates will have a large k_{cat} and low K_m (tight binding) value and hence, a large k_{cat}/K_m value. At low substrate concentrations ($S << K_m$), the enzymatic reaction is first-order (with E_o constant), with the rate constant equal to $(k_{cat}/K_m)E_o$. Hence, at a given enzyme level, E_o, k_{cat}/K_m determines the reaction rate at a low substrate concentration.

During enzyme kinetic experiments and subsequent analysis of data, care must be taken to ascertain that the assumptions of the Michaelis-Menten equation are met. For some substrates experiments have to be carried out under conditions of approximately equal substrate and enzyme concentrations. This approach violates one basic assumption of the derivation of the Michaelis-Menten equation (i.e., $S_o >> E_o$). Dixon [24] has described an elegant and simple direct plot for determining K_m when a substantial fraction of the substrate is bound.

The velocity at any [S] is given by $v = k_{cat}[ES]$. At some given point on the velocity curve, one can write:

$$v = V_{max}\frac{n-1}{n} \tag{3}$$

where n is a whole number. Therefore, at the given point on the velocity curve:

$$k_{cat}[ES] = V_{max}\frac{n-1}{n} \tag{4}$$

$$K_m = \frac{[E]\ [S]}{[ES]}\ \frac{([E]_o - [ES])\ ([S]_o - [E])}{[ES]} \tag{5}$$

After making appropriate substitutions, one obtains:

$$K_m = \frac{[S]_t}{n-1} - \frac{[E]_t}{n} \tag{6}$$

A series of lines are drawn from the origin through points on the velocity curve where $v = V_{max}[(n-1)/n]$. Since n is a whole number, the points correspond to $1/2\,V_{max}$, $2/3\,V_{max}$, $3/4\,V_{max}$, etc. Each line intersects a horizontal line of height V_{max} at different $[S]_n$ values, called $[S]_2$ for the line through $1/2\,V_{max}$, $[S]_3$ for the line through $2/3\,V_{max}$, and so on. The value of each $[S]_n$ is given by:

$$[S]_n = \frac{n}{n-1}[S]_t \tag{7}$$

where $[S]_t$ is the total substrate concentration required for a given n. Consequently:

$$[S]_t = \frac{n-1}{n}[S]_n \tag{8}$$

and from Eqn. 6:

$$K_m = \frac{[S]_n}{n} - \frac{[E]_t}{n} \tag{9}$$

or:

$$[S]_n = nK_m + [E]_t \tag{10}$$

The intercepts on the V_{max} line occur at increments of K_m, i.e.:

$$[S]_n - [S]_{n-1} = K_m \tag{11}$$

This procedure requires a knowledge of approximate V_{max}. It was shown [25] that if the V_{max} value chosen is too low, the distances between the intercepts (i.e., K_m) decrease towards the right. If the V_{max} value chosen is too high, the intervals increase toward the right. Thus, the method gives a check on the assumed value of V_{max}. As a first approximation the V_{max} value obtained from the Lineweaver-Burk plot may be used. An example of this method is shown in Figure 2.

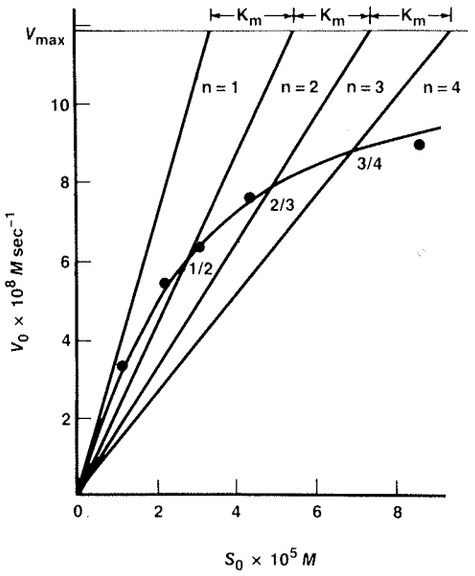


Fig. 2. Dixon plot for determining K_m for the hydrolysis of III by 1.5×10^{-5} M α-chymotrypsin at pH 8.0.

In considering this problem, it is of interest to examine the rate laws which can be obtained from the mechanism of Eqn. 1 without making any assumption about the relative magnitudes of the enzyme and substrate concentrations. Using only the steady-state assumption, solution of this mechanism yields [26]

$$v_o = \frac{k_2}{2} \times ((K_m + S_o + E_o) - \sqrt{(K_m + E_o + S_o - 4E_oS_o)}) \tag{12}$$

in which the symbols are defined as given above. After rearranging, the term under the radical may be modified using the binomial expansion to obtain

$$v_o = \frac{\psi k_2}{2}\left\{\frac{1}{2}\left(\frac{4E_oS_o}{\psi 2}\right) + \frac{1}{8}\left(\frac{4E_oS_o}{\psi 2}\right)^2 + \frac{3}{48}\left(\frac{4E_oS_o}{\psi 2}\right)^3 + \ldots\right. \tag{13}$$

where $\psi = (E_o + S_o + K_m)$. If both E_o and S_o are much less than K_m, Eqn. 13 reduces to

$$v_o = (k_2/K_m)\, E_oS_o \ldots \tag{14}$$

which predicts that, under these conditions, reaction velocities will be first order with respect to both enzyme and substrate concentrations even if $E_o \simeq S_o$.

Let us consider some further properties of Eqn. 13. In particular, we require an expression for the error involved when a system which actually obeys the quadratic form of Eqn. 12 is interpreted in terms of the Michaelis-Menten form of Eqn. 2. Let v_m and v_p be the velocities calculated using Eqns. 2 and 13, respectively; then, if we define the error, E, with respect to v_m and set $x = 4E_oS_o/(K_m + E_o + S_o)^2$, we may derive

$$E = \frac{1}{4}x + \frac{1.3}{4.6}x^2 + \frac{1.3.5}{4.6.8}x^3 + \frac{1.3.5.7}{4.6.8.10}x^4 + \ldots \tag{15}$$

which can be expressed as

$$E = \sum_{i=1}^{\infty} \frac{\prod_{J=1}^{i}(2J-1)}{\prod_{J=1}^{i}(2J+2)}\, x^i \ldots \tag{16}$$

The quantity E is the error introduced if a Michaelis-Menten system in which the enzyme and substrate concentrations are approximately equal is treated as though $S_o >> E_o$. If E_o is held constant, Eqn. 16 yields a maximal E at $S_o = K_m + E_o$.

Another difficulty in treating the data obtained from enzyme kinetic studies arises when the enzyme undergoes inhibition by one of the products of the reaction. Product inhibition of carboxypeptidase has been reported [4, 27, 28]. The results of six runs measuring the hydrolysis rates of aspirin phenyllactic acid (II) at six different concentrations catalyzed by carboxypeptidase A at pH 7.5 are illustrated in Figure 3. The points shown were taken from the continuous record of the titrator for calculation purposes. The curves of this plot do not seem to follow a simple kinetic order, and the sharp decrease of hydrolysis rates with time suggests that product inhibition of the enzyme plays an important role. Significant product inhibition of carboxypeptidase A was reported [5, 6].

The scheme that fits the kinetics of the carboxypeptidase A-catalyzed hydrolysis of II is shown in Scheme 1 (E is carboxypeptidase A, S is II, P_1 is aspirin, and P_2 is L-phenyllactic acid). The L-phenyllactic acid (P_2) produced during hydrolysis acts as a competitive inhibitor.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_{cat}}{\rightleftharpoons} E + P_1 + P_2$$

$$E + P_2 \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} EP_2$$

Scheme 1

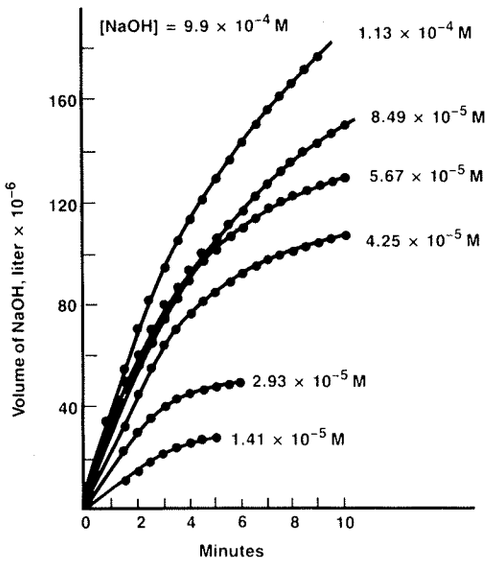


Fig. 3. Plot of volume of sodium hydroxide consumed versus time for the hydrolysis of II by 2.45 × 10^{-10} M carboxypeptidase A at pH 7.5.

The corresponding integrated Michaelis-Menten equation is:

$$\frac{[S]_o - [S]}{t} = \frac{K_m (K_i + [S]_o}{(K_m - K_i)t} \ln ([S]_o/[S])$$

$$+ \frac{V_{max}}{1 - K_m/K_i} = \frac{a}{t} \ln \frac{[S]_o}{[S]} + \frac{V_{max}}{1 - K_m/K_i} \qquad (17)$$

where:

$$K_m = \frac{k_{-1} + k_2}{K_1}, \qquad K_i = \frac{k_{-i}}{k_i}, \qquad a = \frac{K_m (K_i + [S]_o)}{K_m - K_i}$$

The integrated Michaelis-Menten equation for competitive product inhibition applies satisfactorily to the kinetic data obtained at pH 7.5 for II. This finding is illustrated by Figure 4, in which $([S]_o - [S])/t$ is plotted versus $1/t \ln ([S]_o/[S])$. The linearity of the plots obtained is consistent with the proposed competitive product inhibition since the lines would curve for other types of product inhibition.

The intercept of the ordinate of Figure 4 is a function of V_{max}, K_m and K_i, and the slopes of the straight lines, a, are related to K_m, K_i, and $[S]_o$ by:

$$[S]_o = a \left(1 - \frac{K_i}{K_m}\right) - K_i \qquad (18)$$

Thus, to separate the various kinetic parameters, Figure 5 plots the values of $[S]_o$

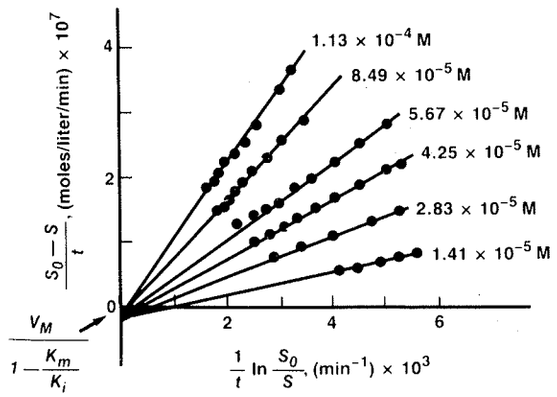


Fig. 4. Plot of integrated Michaelis-Menten equation for competitive product inhibition for the hydrolysis of II by 2.45 × 10^{-10} M carboxypeptidase A at pH 7.5.

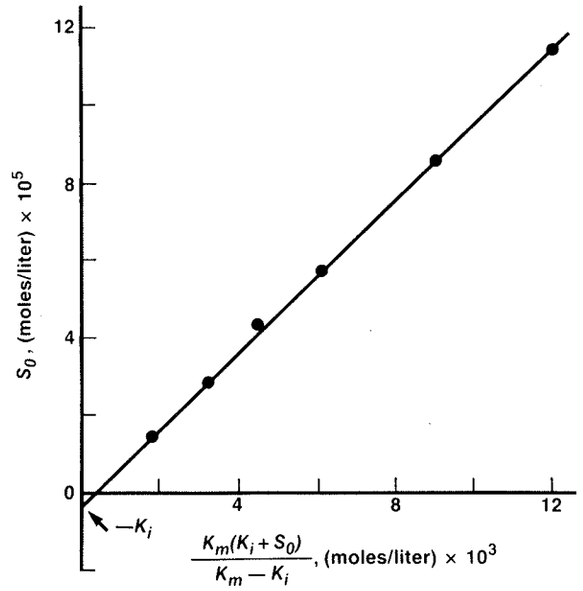


Fig. 5. Plot to obtain K_m and K_i from integrated Michaelis-Menten equation.

versus the respective slopes, a, from Figure 4. The intercept along the $[S]_o$ axis is $-K_i$; hence, K_m and V_{max} can be calculated. The K_i value was considerably smaller than K_m, indicating strong competitive inhibition by the product, L-phenyllactic acid.

4. Prodrug considerations

The most frequently modified functionalities, X, in drug molecules are carboxyl, hydroxyl and amino groups. In developing general guidelines for synthesizing a prodrug D-X-R, the following considerations apply: (i) the prodrug must be sufficiently stable in the dosage form and (ii) D-X-R must be converted rapidly (or at a controlled rate) to D-X in vivo. The objective in introducing an R group is to improve the pharmaceutical/biological properties of the drug. Properties such as solubility, stability, absorption rate, taste, pain on injection, etc., can be modified by the addition of an appropriate R group to the drug. The choice of R from this point of view is not within the scope of this paper, nor is the choice of R for 'active site direction'. The focus is, instead, on the enzymes which may be active in the bio-reconversion of D-X-R to D-X. The hydrolytic enzymes represent the natural target for this reconversion step. We shall review the type of reaction, specificity and potential prodrug substrates for the following enzymes: α-chymotrypsin, trypsin, elastase,

carboxy- and aminopeptidases, carboxylic acid esterases, lipases and phosphatases. Two questions will remain foremost in these discussions: (i) which R groups confer high reconversion rates and (ii) what is the effect of D on the reconversion of D-X-R. Clearly, in order for general suggestions to be made, the high reconversion rates introduced by and dependent on the R group should be little affected by the presence of D.

5. Gastrointestinal enzymes

In discussing the location of intestinal enzymes as prodrug reconversion sites, the gastrointestinal (GI) tract may be viewed as a cylindrical tube. The ability of a drug or prodrug to get to sites of absorption along the tube wall is a function of its GI residence time (how long it takes the drug to travel in an axial direction down the tube) and its radial transport time (how long it takes a drug molecule to move from a point in the tube lumen through the tube wall).

In addition to the pH gradient that exists in the axial direction down the GI tract, the distribution, activity and specificity of enzymes involved in digestion and nutrient transport are very much a function of axial position. For specific enzyme systems, the axial distribution pattern is also a function of species, age, and nutritional state.

Prodrug or drug transport in the radial direction occurs from the lumen, through the mucin layer and glycocalyx at the cell surface, the brush border apical membrane of the enterocyte, the cytosol and basal cell membrane, and through the capillary or lymphatic endothelium (Fig. 6). The radial enzyme distribution pattern in the in-

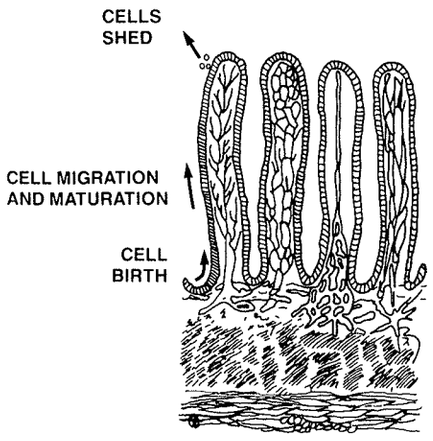


Fig. 6. Diagrammatic section of the wall of the human small intestine. The villi show separately, from left to right, venous drainage, arterial blood supply, lymphatic drainage and nerve supply.

testinal lumen, glycocalyx, brush border membrane and enterocyte cytosol is geared to couple sequential digestion and transport in the process of nutrient absorption. Since the radial regions consist of a series of aqueous and membrane components, the site for prodrug reconversion is critical for successful drug transport and subsequent absorption from the small intestine.

The target choice for prodrug reconversion is dictated by drug physical property transport limitations and enzyme distributions, activities, and specificities. From a pharmacokinetic standpoint, the rate of absorption is roughly dependent on radial considerations, while the extent of absorption is a function of axial parameters.

5.1. Luminal enzymes

Prodrug reconversion in the intestinal lumen can be utilized as an oral drug delivery strategy when a drug has poor stability at acidic pH or causes gastric irritation. In this regard, a prodrug can be made that is stable in the pH range of the stomach and undergoes hydrolysis to the parent drug at intestinal pH. Alternatively, prodrugs can be targeted for the pancreatic enzymes in the intestinal lumen. These enzymes include pancreatic amylase, pancreatic lipase, elastase, trypsin, α-chymotrypsin and carboxypeptidase A [2, 3, 4, 29].

Prodrugs made to increase drug aqueous solubility can be targeted for luminal enzymes in order to gain an advantage with respect to the dissolution rate from a solid oral dosage form. However, luminal reconversion after dissolution regenerates the limitations on aqueous transport of the poorly soluble parent drug. In such cases, it is advisable to target these prodrugs for enzymes associated with the intestinal brush border membrane. These enzymes include adsorbed pancreatic enzymes and enzymes synthesized in the enterocytes which are active at the cell brush border upon maturation at the villus tip.

5.2. Mucosal cell enzymes

Those enzymes synthesized in the enterocyte and associated with the apical brush border membrane include the disaccharidases, aminopeptidases, some carboxypeptidases and alkaline phosphatase. These enzymes are more firmly associated with the brush border membrane than adsorbed pancreatic enzymes [30]. The disaccharidases penetrate the first lipid layer of the membrane and can be released from membrane vesicles by the action of papain. They are subject to attack by the luminal pancreatic proteases, and as a result the time span of disaccharidase-membrane association is only a few hours. Alkaline phosphatase extends the depth of the bilayer while the aminopeptidases penetrate the bilayer and protrude into the microvillus core. Extraction of aminopeptidase and alkaline phosphatase from membrane vesicles requires the application of a detergent to rupture bonds between lipid molecules. These enzymes are glycoproteins in which the protein component

of the enzyme penetrates the membrane lipid matrix while the carbohydrate portion extends out into the lumen, forming part of the outer membrane glycocalyx [30].

Targeting of prodrugs for these enzymes permits prodrug aqueous transport advantages to be maintained up to the intestinal wall. Reconversion at this radial position releases the more membrane-permeable parent drug at a point adjacent to the enterocyte membrane (Fig. 7). A special transport advantage for phosphate and amino acid prodrugs may be gained if enzyme binding and catalytic sites are located so as to permit a favorable positioning of the drug molecule for membrane permeation. Similar transport advantages have been demonstrated in physical systems, where a surface reaction is coupled to diffusion between two phases [31].

In the intact prodrug can pass intestinal membranes, additional radial reconversion sites become available in the enterocyte cytosol or in the bloodstream. The fact that some of the cytosolic aminopeptidases possess different specificities and activities than brush border aminopeptidases and pancreatic proteases provides a potential refinement for this prodrug delivery approach [32, 33]. A brief review of the pertinent active site structure and substrate specificities of some of the targetable enzymes are given below. A more detailed review has been published previously [1].

5.3. Chymotrypsin, trypsin and elastase

5.3.1. Chymotrypsin

Chymotrypsin is an endopeptidase involved in protein digestion. Its inactive precursor, chymotrypsinogen, is produced in the pancreas and converted to α-

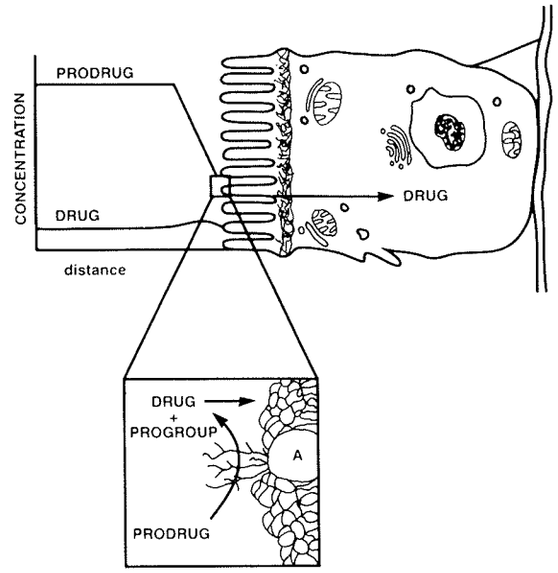


Fig. 7. Proposed mechanism for improving oral absorption.

chymotrypsin (α-CT) in the duodenum by tryptic and, subsequently, chymotryptic action [34].

The molecular weight of α-CT is approximately 25,000 g/mole. The molecule consists of three peptide chains and a total of 241 amino acid residues whose sequence is known completely [35 – 38]. Based on X-ray diffraction studies [37, 39, 40], atomic coordinates for the entire molecule have been published [41]. Blow [38] has reviewed the numerous X-ray studies which focused on the active site region of the native enzyme as well as enzyme-virtual substrate and enzyme-inhibitor complexes.

α-CT catalyzes the hydrolysis of a rather broad spectrum of peptides, amides and esters (see Fig. 8). An absolute requirement of substrate candidates is that the amino-acid residue circled in Figure 8 be of the L configuration. Substrates having an aromatic residue (e.g., Try, Tyr, Phe) are hydrolyzed at appreciable rates [42] (see Table 5), but the hydrolysis of substrates having hydrophobic residues (e.g.,

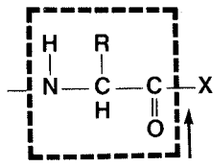


Fig. 8. α-CT catalyzes the hydrolysis of the indicated bond. -X- represents either -O- or -NH-.

TABLE 5
Kinetic Parameters for α-Chymotrypsin-catalyzed Hydrolysis

Substrate	K_m (mole/liter)	k_{cat} (sec^{-1})	k_{cat}, K_m (moles second/liter)	pH
Aspirin phenylalanine ethyl ester (I)	1×10^{-6}	2.92	2.91×10^{6}	7.5
N-Acetyl phenylalanine ethyl ester[a]	1.3×10^{-6} 1.2×10^{-3} [a]	25 160[a]	1.92×10^{7} 1.33×10^{5} [a]	8.0 7.8
Aspirin phenyllactic ethyl ester (III)	0.88×10^{-3} [a] 2.02×10^{-5}	63[a] 7.93×10^{-3}	7.16×10^{4} [a] 3.96×10^{2}	6.99 8.0
O-Acetyl phenyllactic ethyl ester[b]	2.5×10^{-2} 2.3×10^{-2}	3.7×10^{-3} 0.6	1.48×10^{2} 26.1[b]	7.5 8.0
Aspirin phenylalanine amide (II)	5.1×10^{-4}	5×10^{-3}	9.8	7.5
N-Acetyl phenylalanine amide[a]	2.4×10^{-4} 3×10^{-2} [a]	6.2×10^{-3} 4.6×10^{-2} [a]	24.7 1.53[a]	8.0 7.9

a. Taken from Ref. 49.
b. Taken from Ref. 50.

Leu, Met) is also catalyzed [43]. Simple esters, such as *p*-NO_2-phenyl acetate, are also substrates. The enzyme is inhibited by a variety of aromatic compounds, the aromatic amino acids, and the D configuration of many substrates.

Kinetic studies have revealed a bell-shaped relationship between the overall reaction rate and pH, maximum activity occurring near pH 8 [44]. One can distinguish, kinetically, between three groups of substrates: specific amide substrates (i.e., derivatives of the aromatic amino acids, or similar compounds, wherein a peptide bond is cleaved), specific ester substrates, and non-specific substrates with good leaving groups (e.g., *p*-NO_2-phenyl acetate). The following scheme [45, 46] seems to apply to all three classes [47 – 49]:

$$E + S \overset{K_s}{\rightleftharpoons} E - S \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E \xrightarrow[P_1]{+} P_2 \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E + P_2$$

The three reaction steps represent the formation of the non-covalent (Michaelis) enzyme-substrate complex, the formation of an acyl-enzyme with the loss of the leaving group, and the deacylation step. For non-specific substrates with good leaving groups [50] and for specific ester substrates [44, 45], the deacylation step is rate-limiting, whereas for specific amide substrates [51, 52] the rate is determined by the acylation step.

Kinetic studies combined with high-resolution X-ray investigations have provided a relatively complete understanding of the binding and catalytic mechanisms of α-CT. Many of the papers treating the binding mechanisms have been reviewed by Cunningham [42]. The scheme illustrated in Figure 9 is an oversimplification, but it provides a rationale for many of the observed substrate specificities as well as a basis for discussions of the catalytic mechanism. Four binding sub-sites are arranged approximately tetrahedrally. The aryl site (ar, p_2) binds the side chain and is responsible for the preference of aromatic substrates. The acylamide site (am, p_1) (carbonyl oxygen of Ser 214) may form a H-bond with the acylamide group of the substrate and, thereby, hold the molecule in a position favorable for hydrolysis. The hydrolytic site (n, p_3) consists, primarily, of the Ser 195 side chain whose alcoholic

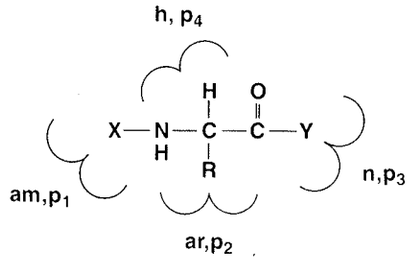


Fig. 9. The four binding subsites of α-CT illustrated with a typical substrate.

oxygen forms a covalent bond with the substrate's carbonyl carbon atom. The hydrogen (h, p_4) site was originally thought to be a site of restricted volume since replacement of the α-hydrogen with a methyl group renders the molecule ineffective as a substrate [53 – 55]. However, no h site is actually observed and it is now believed that restrictions on the size of the α-carbon substituent are the result of steric restrictions within the substrate itself [38].

It is interesting to contrast the catalytic mechanism of α-CT with that of carboxypeptidase A or leucine aminopeptidase (see below). In the latter cases, the carbonyl bond of the scissile peptide bond is polarized by a metal ion, making the carbon atom more susceptible to nucleophilic attack. In α-CT, the complementary approach is taken: the negative charge and, hence, the nucleophilicity of the attacking Ser 195 oxygen is significantly increased by the 'charge relay system' [37] illustrated in Figure 10. Blow [56] has reviewed the catalytic mechanism of α-CT in considerable detail.

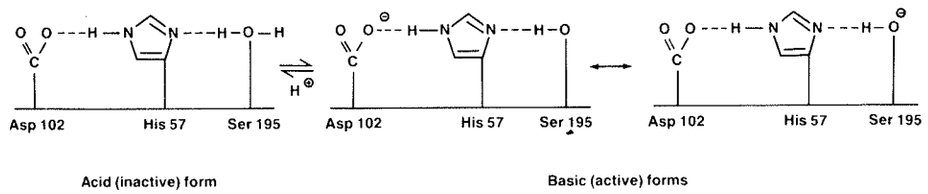


Fig. 10. 'Charge relay system' of α-CT.

5.3.2. Trypsin

Trypsin is a proteolytic enzyme produced in the pancreas as the inactive precursor, trypsinogen. Trypsinogen is a single-chain molecule weighing 23,800 g/mole and is composed of 229 amino acid residues cross-linked by six disulfide bridges. Activation of trypsinogen to beta-trypsin results from scission of the peptide bond joining residues 6 and 7 and may be accomplished either by auto-catalysis in alkaline medium or by the action of extracellular proteolytic enzymes. Subsequent degradation of beta-trypsin has been shown to produce additional active forms of trypsin [57].

The crystal structures of beta-trypsin and bovine pancreas inhibitor have been determined by X-ray diffraction studies [58, 61]. Further studies (at 1.8 Å resolution) have established the atomic coordinates of the beta-trypsin-benzamine complex [62]. Comparison of the three-dimensional structures of α-CT and trypsin reveals almost complete homology, the major differences being the replacement of a serine at the bottom of the binding site pocket in α-CT with an aspartate group in trypsin. This accounts for the observed preference of trypsin for the basic L-amino acids arginine and lysine.

In other respects (e.g., pH profile, mechanism, etc.) trypsin is very similar to α-CT [59]. Table 6 presents some of the kinetic data for several substrates.

TABLE 6
Kinetic Parameters for Various Substrates of Beta-Trypsin

Substrate	$K_m \times 10^3$ M	k_{cat} (sec^{-1})	pH	Reference
N-Tosyl-L-arginine methyl ester	0.0064	75	8.4	63
N-Tosyl-L-homo-arginine methyl ester	0.330	4.04	8.4	63
N-Benzoyl-L-lysine methyl ester	0.017	16.6	8.0	64
N-Tosyl-L-lysine methyl ester	0.042	67.2	8.0	64

5.3.3. Elastase

Elastase is a serine protease composed of a single chain of 240 amino acid residues cross-linked by four disulfide bridges. Elastase is produced by the activation of proelastase and has a molecular weight of approximately 25,000 g/mole.

The three-dimensional structure of elastase has been determined by X-ray crystallographic studies and is also quite homologous to the structure of α-chymotrypsin [65, 66].

Elastase, so named because it was originally thought to hydrolyze only elastin, actually has a broad specificity that complements the other pancreatic enzymes, α-chymotrypsin and trypsin.

Elastase is specific toward peptide bonds involving the carbonyl group of amino acids bearing uncharged, non-aromatic side chains. These amino acids include glycine, alanine, valine, leucine, isoleucine and serine [67]. Elastase also hydrolyzes esters and amides [68]. The esterase, amidase and peptidase activity of elastase has been shown to increase with the length of the leaving group [68], suggesting a more important role for the secondary binding sites in the catalytic activity of this enzyme compared to α-CT and trypsin. While substrates with bulky aromatic side chains are hydrolyzed by α-chymotrypsin and trypsin, elastase shows no activity with these

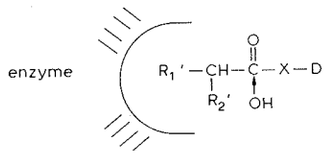
TABLE 7
Kinetic Parameters for Elastase

Substrate	K_m (mM)	k_{cat} (sec^{-1})	Reference
Ala-Ala-*O*-nitro benzyl ester	14.3	61	60
Ala-Ala-Ala-*O*-nitro benzyl ester	0.167	67	60
Ala-Ala-Ala-*O*-methyl ester	15	40	58
Ala-Ala-Ala-Ala-*O*-nitro benzyl ester	0.042	77	60
Ala-Ala-Ala-Ala-*O*-methyl ester	0.25	50	60
Ac-Pro-Ala-Pro-Ala-Gly-NH_2	4.0	26	62
Ac-Ala-Ala-Ala-HN_2	2.5	0.38	58
C_6H_5-CO-Ala-OCH_3	13	23	61
C_6H_5-CO-Ala-OC_4H_9	2.7	24	61

substrates. These differences in specificity are explained by structural differences at the binding site. In elastase, the pocket is partially occupied by a threonine residue and partially occluded by a valine residue [69]. Hence, elastase is specific for smaller, unbranched, non-aromatic side chains. In other respects elastase is quite similar to α-CT and trypsin. Typical kinetic parameters are given in Table 7.

5.3.4. Prodrug considerations

Since these enzymes are primarily active in the GI tract, prodrugs designed with these enzymes as reconversion sites are, clearly, restricted to predominantly oral dosage forms. A general substrate for these enzymes is of the form $R_1-\overset{\overset{O}{\|}}{C}-X-R_2$, where X is O or N – H. For these enzymes to be active, R_1 must be the active site-directing group while R_2 would be the drug moiety. Clearly, the most direct application of this approach would be to drugs with free hydroxyl or amino groups. The most obvious procedure would be to make amino acid derivatives of the form



where D is the drug moiety and R_1' the appropriate amino acid side chain. Table 8 presents some general suggestions. The R_2' groups may be, for example, a hydrogen, a free amino group or an acetylated amino group. The acetylated amino group would probably give faster reconversion rates, but other groups may provide sufficient rates for most purposes. In general, the effect of D on the reconversion rate is small except, possibly, for elastase, where the binding site pocket is small and the leaving group

TABLE 8
Prodrug Suggestions for α-Chymotrypsin, Trypsin and Elastase

Reconversion site (enzyme)	R_1'	Utility
α-Chymotrypsin	Phenylalanine, tyrosine, tryptophan or analogues	Reduce polarity, reduce solubility
Trypsin	Lysine, arginine or analogues	Increase solubility, convert drug into weak base, prepare a salt
Elastase	Glycine, valine, leucine, isoleucine or analogues	Reduce polarity, reduce solubility

binding site plays a larger role. One final point to note is that if D and R_1' are similar, the prodrug may bind with D in the pocket ('wrong-way binding') and act as its own competitive inhibitor, thereby reducing reconversion rates.

5.4. Carboxy- and Aminopeptidases

5.4.1. Carboxypeptidase A

Carboxypeptidase A (CPA) is a pancreatic hydrolase whose primary function is in protein digestion. It is produced as an inactive precursor which is converted to the active form by the action of trypsin [72].

Having a molecular weight of approximately 34,600 g/mole, the active enzyme consists of a single chain of 307 amino acid residues and one zinc ion. The amino acid sequence is known almost completely [73, 74] and the three-dimensional structure has been investigated in several laboratories [75 – 79]. Since the crystal has been shown to be enzymically active [80], the high-resolution (2 Å) studies on the native enzyme [77 – 79] and the enzyme-Gly-L-Tyr complex [78] have been particularly useful in elucidating the binding and catalytic mechanism.

The zinc ion has been shown to be essential for catalytic activity even though its removal does not greatly affect the structure of the enzyme [81]. The enzyme remains active after the Zn^{2+} ion has been replaced by Fe^{2+}, Mn^{2+}, Co^{2+} or Ni^{2+} [82, 83] and, under certain conditions, the Co-CPA is a more efficient catalyst than Zn-CPA [83]. In the native enzyme, the four Zn^{2+} ligands are the side chains of His 69, His 196, Glu 72, and a water molecule while, in the enzyme-substrate complex, the carbonyl oxygen of the scissile peptide bond replaces the water as the fourth ligand (see discussion below). The primary action of CPA is, as shown in Figure 11, that of a C-terminal exopeptidase. The enzyme also functions as an esterase, but relatively few studies of this activity have been made (e.g., Ref. 84). There are two absolute requirements for potential CPA substrates [85, 86]: the terminal carboxyl group must be free and the C-terminal residue must be of the L configuration. CPA will catalyze the hydrolysis of almost any peptide meeting the above requirements, although substrates wherein R is a branch aliphatic or, optimally, an aromatic group are favored significantly. The penultimate residue may be merely an acyl group, although the rate of catalysis is moderately enhanced if R_1 is an aromatic group. The rate of catalysis is significantly reduced if R_2 is an aromatic group, if R_1 is an acidic group, or if the substrate is a dipeptide with a free amino group. The data in Table 9 illustrate

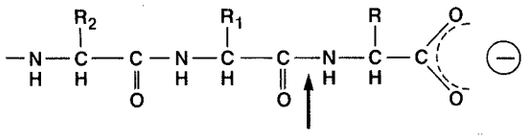


Fig. 11. CPA catalyzes the hydrolysis of the indicated peptide bond.

TABLE 9
CPA-catalyzed Hydrolysis of Some Acylated Dipeptides

Substrate[a]	C_1[b]
CBO-Gly-Phe	13.0
CBO-Gly-Tyr	7.0
CBO-Gly-Try	4.7[c]
CBO-Gly-Leu	2.6
CBO-Gly-Met	1.2
CBO-Gly-Ile	0.54
CBO-Gly-Ala	0.038
CBO-Gly-amino isobutyric acid	0.013
CBO-Gly-Gly	0.0024
CBO-Ala-Phe	11.0
CBO-Ala-Tyr	6.3
CBO-Glu-Phe	0.8
CBO-Glu-Tyr	0.5
CBO-Try-Ala	0.12[c]
CBO-Try-Gly	0.0068[c]
CBO-Try-Pro	0.0025

Taken from an early review by Smith and Hill [97].

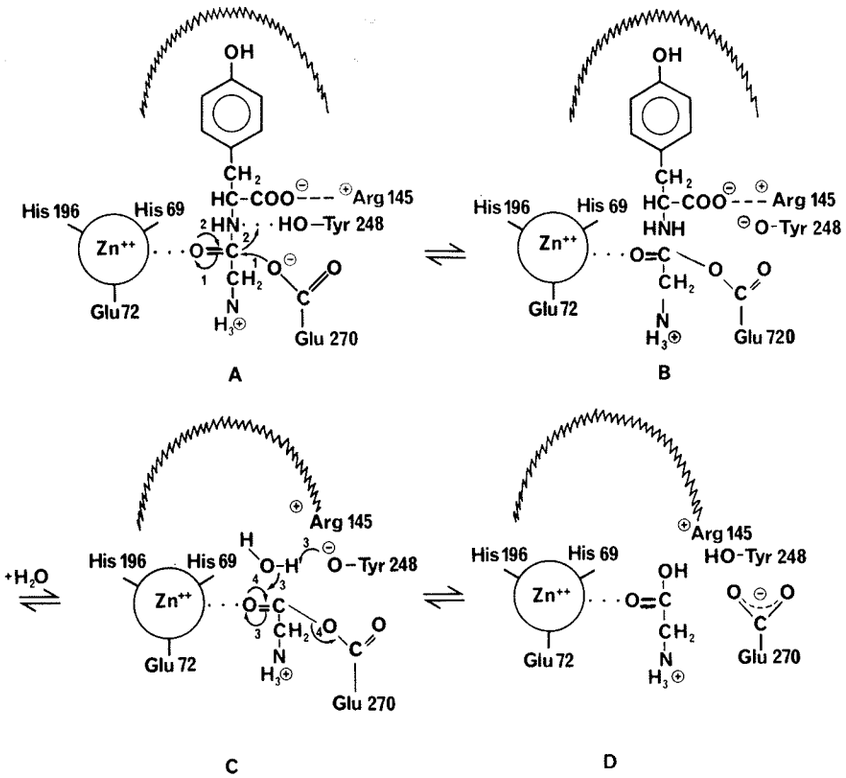
a. CBO = carbobenzoxy, all optically active residues are L-configurations.

b. C_1 is the first-order proteolytic coefficient obtained for 0.05 M substrate at 25°C and pH 7.5 ± 0.2.

c. Compounds demonstrated zero-order kinetics – C_1 computed from initial rate of hydrolysis as if it were first-order.

some of the above generalizations. As previously noted, the terminal residue may also be an α-hydroxy-acid (e.g., benzoylglycyl-L-phenyl-lactic acid is a good substrate). In general, amides are hydrolyzed faster than the homologous esters. Molecules having a carboxylate group two carbons away from an aromatic ring act as competitive inhibitors (e.g., beta-phenyl propionic acid).

The following mechanism [70, 71, 81, 88] is consistent with most existing data and provides a basis for the observed substrate specificities. The Michaelis complex is stabilized by the following interactions:



(i) The R group interacts non-specifically with a large hydrophobic pocket, displacing at least four water molecules from that area.
(ii) The terminal carboxylate group interacts with the positively charged guanidinium group of Arg 145, which moves about 2 Å to improve the interaction.
(iii) The Tyr 248 side chain moves about 14 Å to put the -OH near the scissile peptide bond.
(iv) The carbonyl oxygen of the peptide bond replaces water as the fourth Zn^{2+} ligand.
(v) The carboxylate of Glu 270 may interact with the amide group of the penultimate residue either directly, if the substrate is a protonated dipeptide, or by H-bonding through a water molecule.

It is worth noting that the enzyme distortions mentioned in (ii) and (iii) above make CPA one of the best known examples of Koshland's 'induced fit' theory [88, 89].

Interaction with the Zn^{2+} ion polarizes the carbonyl bond, making the carbon atom more susceptible either to nucleophilic attack by a carboxylate oxygen of Glu 270 or to the Glu 270-facilitated attack by H_2O in a general base-type catalysis. Although an acyl-enzyme intermediate is formed by the first attack mechanism and