Drugs of the Future 1991, 16(5): 443-458

Review Article

Novel chemical approaches in prodrug design

Hans Bundgaard
Royal Danish School of Pharmacy,
Department of Pharmaceutical Chemistry,
2 Universitetsparken, DK-2100 Copenhagen, Denmark

CONTENTS

Introduction

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 reach 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.

Prodrugs are designed to overcome pharmaceutically and/or pharmacokinetically based problems associated with the parent drug molecule that would otherwise limit the clinical usefulness of the drug. The prodrug approach can

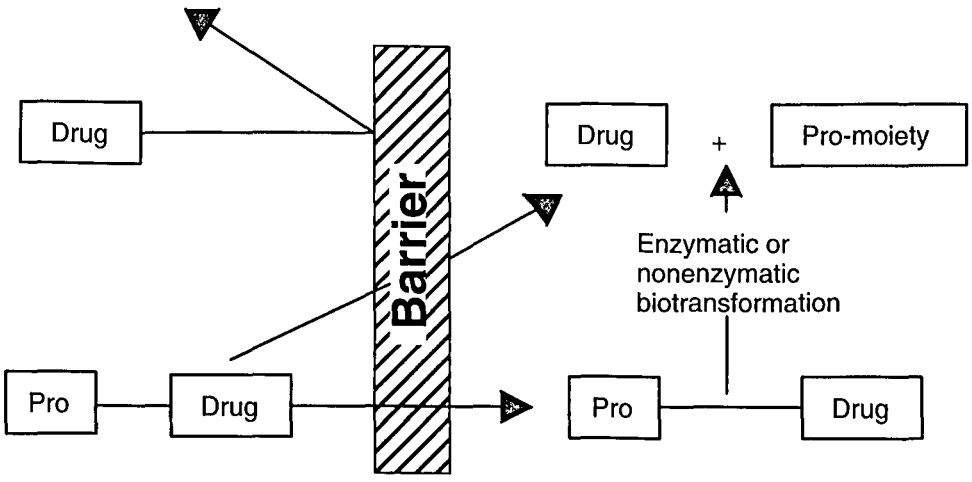


Fig. 1. Schematic illustration of the prodrug concept as a means of improving drug absorption.

be illustrated as shown in Figure 1. The usefulness of a drug molecule is limited by its suboptimal physicochemical properties, *e.g.*, it shows poor biomembrane permeability. By attachment of a pro-moiety to the molecule or otherwise modifying the compound, a prodrug is formed that overcomes the barrier for the drug's usefulness. Once past the barrier, the prodrug is reverted to the parent compound by a post-barrier enzymatic or non-enzymatic process. Prodrug formation can thus be considered as conferring a transient chemical cover to alter or eliminate undesirable properties of the parent molecule.

The prodrug approach has been successfully applied to a wide variety of drugs. Most of the applications have involved: 1) enhancement of bioavailability and passage through various biological barriers, 2) increased duration of pharmacological effects, 3) increased site-specificity, 4) decreased toxicity and adverse reactions, 5) improvement of organoleptic properties, and 6) improvement of stability and solubility properties (1-6).

A basic 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 reached. 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 enzymatic 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 or liver. In other cases, active drug substances are regenerated from their prodrugs by biochemical reductive or oxidative processes.

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. A serious drawback of prodrugs requiring chemical (non-enzymatic) 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 double prodrugs, where use is made of an enzymatic release mechanism prior to the spontaneous reaction.

In recent years several types of bioreversible derivatives have been exploited for utilization in designing prodrugs (7, 8). An account of novel chemical approaches in the design of prodrugs is given in the following.

Ester prodrugs

The popularity of using esters as a prodrug type for drugs containing carboxyl or hydroxyl functions stems primarily from the fact that the organism 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 caboxyl group it is feasible to obtain derivatives with almost any desirable hydro- 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 (7).

Sometimes, however, many aliphatic or aromatic esters are not sufficiently labile *in vivo* to ensure a sufficiently high rate and extent of prodrug conversion. For example, simple alkyl and aryl esters of penicillins are not hydrolyzed to the active free penicillin acid *in vivo* and therefore have no therapeutic potential (9). The reason for this is the highly sterically hindered environment about the carboxyl group in the penicillin molecule which makes enzymatic attack on the acyl group very difficult.

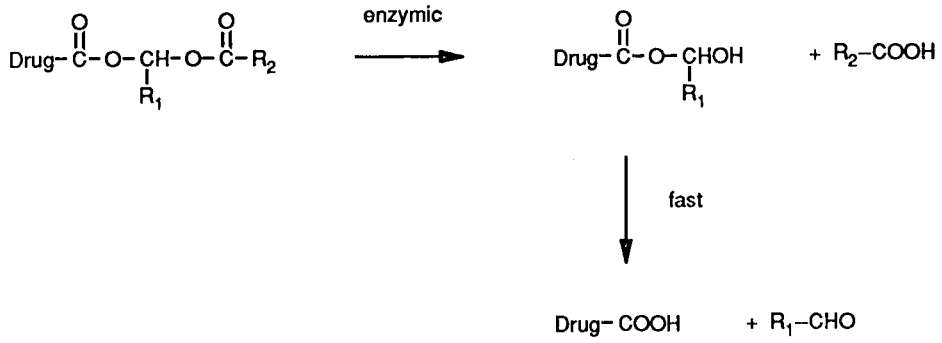
Double esters

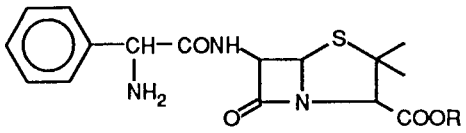
This shortcoming can be overcome by preparing a double ester type, (acyloxy)alkyl or [(alkoxycarbonyl)oxy]alkyl esters in which the terminal ester grouping is less sterically hindered. The first step in the hydrolysis of such an ester is enzymatic cleavage of the terminal ester bond with formation of a highly unstable hydroxymethyl ester which rapidly dissociates to the parent acidic drug and formaldehyde (Scheme 1).

This 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), the ethoxycarbonyloxyethyl ester (**3**) (bacampicillin), and the phthalidyl ester (**4**) (talampicillin) (for a review, see ref. 9). Bacampicillin contains a terminal carbonate ester moiety and releases ethanol, carbon dioxide and acetaldehyde upon hydrolysis. In talampicillin the pro-moiety released upon hydrolysis is 2-carboxybenzaldehyde which is further metabolized to 2-hydroxymethylbenzoic acid.

In more recent years the applicability of this double ester concept in prodrug design has been further expanded. Thus, similar esters have been prepared from various non-steroidal antiinflammatory agents as well as from methyldopa (10), cromoglycic acid (11), furosemide (12) and

Scheme 1



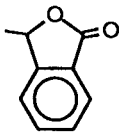


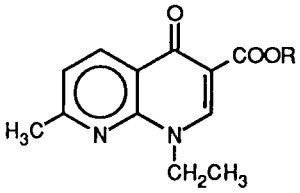
(1) R = H

(2) R = $-CH_2O-C(=O)-C(CH_3)_3$

(3) R = $-CHO(CH_3)-C(=O)-OCH_2CH_3$

(4) R =





(5) R = H

(6) R = $-CH_2O-C(=O)-CH_2CH_2CH_3$

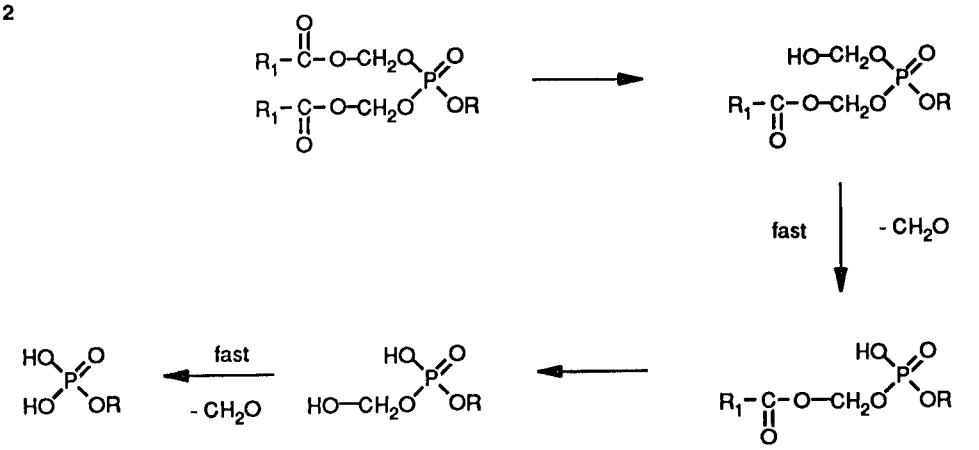
nalidixic acid (13), and found to be useful as prodrugs for enhancement of the dermal or oral delivery of these acidic drugs. The advantage of such esters in terms of enzymatic lability can be illustrated with nalidixic acid (**5**). Whereas the methyl ester shows less than 5% hydrolysis upon incubation in human plasma for 24 h, the butyryloxymethyl ester (**6**) is rapidly hydrolyzed, the half-life being 8 min (13).

The applicability of α-acyloxyalkyl esters as biologically reversible transport forms has been extended to include the phosphate group and phosphonic acids (8). Both the chemical and enzyme-mediated hydrolysis of bis(acyloxymethyl) esters of phosphomonoesters take place as shown in Scheme 2, with the intermediate formation of a monoacyloxymethyl ester (14, 15). The *O*-hydroxymethyl derivatives formed upon ester hydrolysis have only a transitory existence and spontaneously eliminate one molecule of formaldehyde. The bis(acyloxymethyl) ester derivatives are neutral compounds, and they can conceivably traverse cell membranes by passive diffusion and then revert by enzymatic cleavage of the protective group to the parent phosphomonoester. Reports about the application of this prodrug approach to biologically important nucleotides certainly may soon appear.

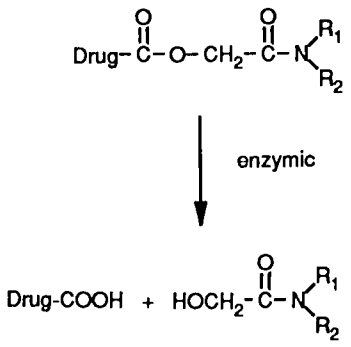
Biolabile glycolamide esters

An alternative solution to the problem of obtaining enzymatically labile ester prodrugs of carboxylic acid agents is provided by *N,N*-disubstituted glycolamide esters. Such esters have recently been shown to be cleaved with remarkable speed in human plasma, the responsible enzyme being pseudocholinesterase (Scheme 3) (16-18). As seen from the examples listed in Table I, such esters derived from various carboxylic acids are hydrolyzed much more facilely than the corresponding simple methyl or ethyl esters. The glycolamide esters combine a high susceptibility to undergo enzymatic hydrolysis in plasma with a high stability in aqueous solution and furthermore, this new ester prodrug type is characterized by providing ample possibilities for varying the water and lipid solubilities of the derivatives with retainment of the favorable enzymatic/nonenzymatic hydrolysis

Scheme 2



Scheme 3



index. One obvious area of application of this ester prodrug type concerns nonsteroid antiinflammatory drugs (19, 20). Esterification of these carboxylic acid agents is known to reduce their gastric ulcerogenic activity. However, simple alkyl esters of these agents are inefficiently cleaved in the organism and are often also highly insoluble in water. In contrast, the glycolamide esters have a high capacity to release the parent active drugs following absorption and possess physicochemical properties favorable for peroral absorption (19).

Table I: Half-lives ($t_{1/2}$) of hydrolysis of esters of various drugs and compounds containing a carboxylic acid function in 80% human plasma (pH 7.4, 37°C) (16, 19).

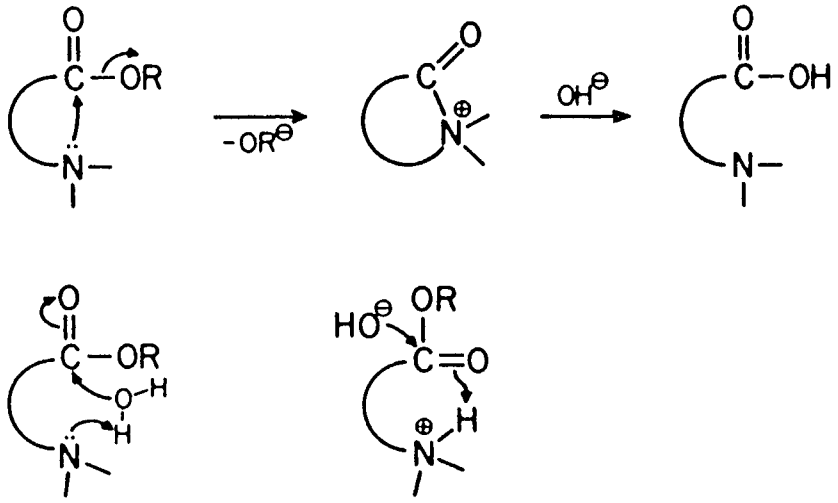
Acid	T1/2	
	Methyl ester	*N,N*-Diethylglycolamide ester
Salicylic acid	17.6 h	0.80 min
4-Aminobenzoic acid	>100 h	0.6 min
Ketoprofen	>20 h	0.5 min
Fenbufen	4.7 h	3.8 min
Tolmetin	19 h	13.4 min
Tolfenamic acid	100 h	5.0 min
Indomethacin	150 h	25 min
Naproxen	20 h	0.6 min
Furosemide	>100 h	4.4 h
Tranexamic acid	4.0 h	1.2 min
L-Tyrosine	1.0 h	0.5 min

Water-soluble ester prodrugs

Formation of water-soluble ester prodrugs has long been recognized as an effective means of increasing the aqueous solubility of drugs containing a hydroxyl group, aimed at developing improved preparations for parenteral or ophthalmic administration. The most commonly used esters for increasing the aqueous solubility of hydroxyl-containing agents are esters containing an ionizable group, *i.e.*, dicarboxylic acid hemiesters, phosphate esters and α-amino acid esters (7). The ideal properties of such prodrugs are as follows: they should possess a high water solubility at the pH of optimal stability and sufficient stability in aqueous solution to allow long-term storage (>2 years) of ready-to-use solutions and yet they should be converted quantitatively and rapidly *in vivo* to the active parent drug. However, none of these derivatives may often fully satisfy all these requirements. Thus, whereas α-amino acid esters or related short-chained aliphatic amino acid esters are in general readily hydrolyzed enzymatically, they exhibit a very poor stability in aqueous solution, making it impossible to prepare ready-to-use solutions (7).

The major reason for the high instability of α-amino and short-chained aliphatic amino acid esters in aqueous solution at pH values affording their favorable water-solubility

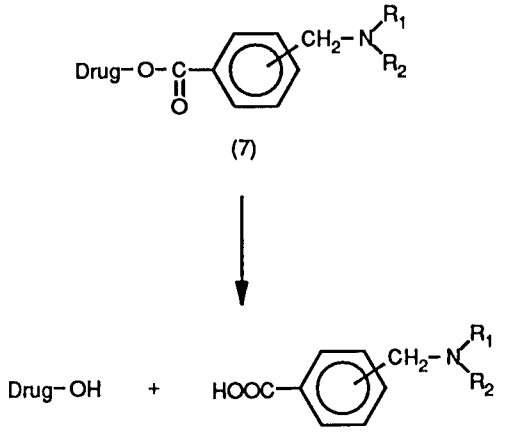
Scheme 4



(*i.e.*, pH 3-5) is partly due to the strongly electron-withdrawing effect of the protonated amino group which activates the ester linkage toward hydroxide ion attack and partly (and predominantly) to intramolecular catalysis or assistance by the neighboring amino group of ester hydrolysis (21, 22). The mechanisms involved include intramolecular nucleophilic catalysis, intramolecular general-base catalysis or general-base specific base catalysis, as depicted in Scheme 4.

It has recently been shown (23) that an effective and simple means to totally block the hydrolysis-facilitating effect of the amino group and yet retain a rapid rate of enzymatic ester hydrolysis is to incorporate a phenyl group between the ester moiety and the amino group. By doing so the intramolecular catalytic reactions of the amino group, as outlined in Scheme 4, are no longer possible for steric reasons and furthermore, the ester-labilizing effect of the protonated amino group due to its polar character is greatly diminished. Because of the requirement of a pK_a value greater than 5-6 for the amino group (for solubility reasons), the group is not directly attached to the phenyl nucleus but separated from this by an alkylene group, in the most simple case a methylene group. Such *N*-substituted 3- or 4-aminomethylbenzoate esters (**7**) have been found to be readily soluble (often >25%) in water at weakly acidic pH values and to possess a very high stability in such solutions combined with a high susceptibility to undergo enzymatic hydrolysis in the presence of plasma (Scheme 5) (23). Thus, the 4-(morpholinomethyl)benzoate ester of metronidazole (**8**) possesses a shelf-life of more than 10 years in aqueous solution of pH 4 and 25°C while being hydrolyzed to metronidazole (**9**) in human plasma with a half-life of 0.4 min (Scheme 6). The pK_a of the morpholino group in compound (**8**) is 6.1 and readily water-soluble salts can be formed with *e.g.*, hydrochloric acid (24). Similar esters with the same favorable solubility, *in vitro* stability and *in vivo* lability characteristics have been described for various corticosteroids (23), chloramphenicol

Scheme 5



(7)

(25), acyclovir (23), ganciclovir and other hydroxyl-containing drugs (23, 26)

These properties regarding solubility, chemical stability and enzymatic lability make *N*-substituted aminomethylbenzoate esters a promising new prodrug type for slightly soluble drugs containing an esterifiable hydroxyl group. In addition to being useful for parenteral or ophthalmic administration, these novel prodrugs may be applied to improve the peroral, rectal or dermal bioavailability of slightly water-soluble drugs. In this regard, it should be noted that the lipophilicity of the prodrug derivatives can readily be modified or controlled by the appropriate selection of the amino group both in terms of amine basicity and hence degree of ionization at physiological pH, and in terms of hydrophobicity of the substituents on the nitrogen atom. It has thus been

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.