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RAPID HYDROLYSIS OF AMIDES UNDER PHYSIOLOGICAL CONDITIONS: INFLUENCE OF THE MICROENVIRONMENT ON THE STABILITY OF THE AMIDE BOND

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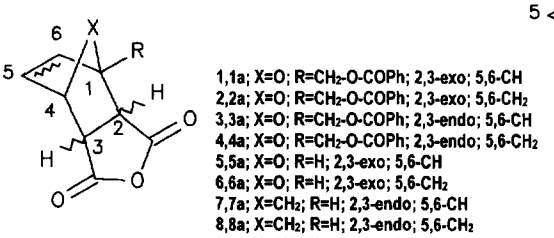
Abstract: A new class of bicyclic carboxyamides **1a-9a** differing with respect to substitution patterns and exo-endo geometry has been synthesized. These amides are characterized by a structure-dependent unusual rapid hydrolysis rate at physiological conditions. The corresponding bicyclic anhydrides might be used as tools for masking and modifiying therapeutic agents containing amine functionalities.

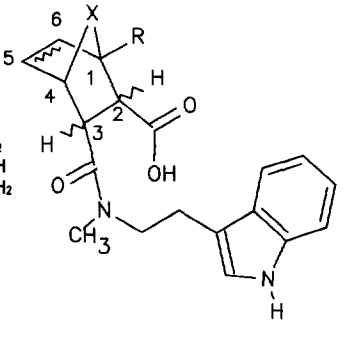
Under physiological conditions the hydrolysis of amides exhibits half-lives in the range of 7 years[1]. However, the intramolecular acid catalyzed amide cleavage of phthalic acid amides[2], maleamic amides[3] and ß-carboxyamides[4] revealed enhanced hydrolysis, initiated by hydrogen ion transfer from the adjacent carboxy group to the amide moiety and followed by intramolecular nucleophilic attack of the neighboring carboxylate ion. Here we present the structure-stability relationships of a new class of very acid-labile amides formed by the reaction of primary or secondary aliphatic amines with bicyclic anhydrides.

The aim of this study was the construction of new prodrugs, that contain tailor-made acid-labile protective groups as masking moieties for active drugs for application in cancer therapy[5]. For that we identified key substituents which have major influence on the acid lability of the amide bond formed between an amine and an anhydride. We created a wide range of amine release rates for biological important amines by

Scheme 1



1,1a; X=O; R=CH_2-O-COPh; 2,3-exo; 5,6-CH
2,2a; X=O; R=CH_2-O-COPh; 2,3-exo; 5,6-CH_2
3,3a; X=O; R=CH_2-O-COPh; 2,3-endo; 5,6-CH
4,4a; X=O; R=CH_2-O-COPh; 2,3-endo; 5,6-CH_2
5,5a; X=O; R=H; 2,3-exo; 5,6-CH
6,6a; X=O; R=H; 2,3-exo; 5,6-CH_2
7,7a; X=CH_2; R=H; 2,3-endo; 5,6-CH
8,8a; X=CH_2; R=H; 2,3-endo; 5,6-CH_2
9,9a; X=CH_2-CH_2; R=H; 5,6-CH_2



means of controlled structural variation of key substituents close to the catalytic center. In order to get a great structural variety we chose highly substituted bicyclic anhydrides as precursors for our acid labile amides.

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A panel of bicyclic anhydrides was synthesized differing with respect to substitution patterns and exo-endo geometry[5,6] and chose N-methyl-tryptamine as a model amine. Scheme 1 shows different bicyclic anhydrides **1-9** and their corresponding N-methyl-tryptamine derivatives **1a-9a** as model amides. The correlation between cleavage rates of the amide bonds and substitution patterns requires kinetic parameters that are independent of the correspondent structure related dissociation constants (K_a), because the cleavage rate is proportional to the degree of protonation of the neighboring carboxy group[2-4]. Under identical reaction conditions, kinetic experiments were thus performed at different pH-values to determine $pK_{(app)}$.The results of these studies are compiled in Table 1. The data revealed that all oxygen-bridged ring systems exhibit comparable pK-values of 4.5-4.7. On the other hand, the amides **7a** and **8a** show pK-values of 5.4, and the amide **9a** exhibits an unusual high value of 5.9[7].

Table 1: Cleavage rates [a] and derived parameters [b] of the N-methyl-tryptamine adducts **1a-9a**

Compound	$pK_{(app)}$	$T_{1/2\ (lim)}$ [min]	$T_{1/2}$ (pH 5.0) [min]	$T_{1/2}$ (pH 6.0) [min]	$T_{1/2}$ (pH 7.0) [h]
1a	4.6	4.2	67	336	39
2a	4.6	1.1	17	106	14
3a	4.7	19.3	312	1560	208
4a	4.5	7.0	155	930	124
5a	4.5	2.5	31	159	21
6a	4.6	0.9	10	52	7
7a	5.4	0.5	2	13	1.7
8a	5.4	0.5	2	13	1.7
9a	5.9	0.1	0.3	0.5	0.04

[a] The cleavage rates of the adducts **1a-9a** were determined by HPLC and UV-spectroscopy at 37°C and 150 mM salt at different pH values (k_{obs}). The half-lives ($T_{1/2} = 0.693/K_{obs}$) were calculated according to *pseudo*-first order kinetics. [b] The kinetic data in the pH-range 3-8 were processed according to the formula[4] $1/k_{obs} = K_a/k_{lim}\ [H^+] + 1/k_{lim}$; K_a represents the dissociation constant; k_{lim} is the rate-constant at 100 % protonation of the corresponding carboxy groups of **1a-9a**, respectively.

The comparison of half-lives under reaction conditions causing complete protonation of the carboxy group ($T_{1/2(lim)}$) clearly shows a strong structure-dependent lability. The exo-compound **1a** is five times more reactive than the corresponding endo-carboxyamide **3a**. In addition, the saturated, oxygen-bridged structures are generally about 2-3 times more labile than the corresponding unsaturated compounds. In contrast to this observations **7a** and **8a** exhibit comparable half-lives. Moreover, substitution of the oxygen by an ethane bridge results in a dramatically increased cleavage rate. The carboxyamide **9a** exhibits an extremly short half-life ($T_{1/2(lim)}$) of only 6.0 sec; even at pH 7.0, the half-life of this amide is only 2.6 min. The compound **9a** is

one of the most labile carboxyamides known[8,9]. The lability of this amide bond is comparable with enzyme-catalyzed reaction rates. Comparison of the hydrolysis rate of **9a** with **3a** ($T_{1/2(lim)}$) revealed that **9a** is 190 times more labile, and more than 3000 times more acid-sensitive at pH 7.0. This pH-dependent difference in the cleavage rate is explained by a higher degree of protonation of the carboxy group of carboxyamide 9**a** in comparison with 3**a**. Due to the high pK_a of the carboxy group, a high fraction of kinetically active protonated carboxy groups is present 9**a**, even under neutral conditions. AM1-calculations[10] indicated, that the interatomic C-C-distances between the carboxy groups and the corresponding amide bonds are structure-dependent. These calculations indicate that the amides **7a** and **8a** are characterized by the same geometric properties. This correlates with the observed very similar pH-dependent cleavage rates (Table 1). The distance of the acid carbonyl carbon from the adjacent amide carbonyl carbon in **9a** was only 2.79 Å, in contrast to 2.94 Å in the far more stable compound **3a**[11]. Obviously, the geometric properties of **9a** are ideally suited for intramolecular, acid-catalysed cleavage of the amide bond. This data show, that only small changes in the geometry of the catalytic center cause dramatic change in the reaction rate[13,14].

On the basis of the present structure-reactivity analyses it is possible to generate a wide spectrum of different acid-labilities by variation of key substituents of the bicyclic ring system. Besides their application as protective groups in organic synthesis, new strategies may be envisaged for the tailoring of natural and synthetic compounds for therapeutic use. Many important drugs contain amino functionalities that are crucial for their biological activity. Due to the chemical flexibility inherent in the approach described here, agents of widely differing chemical structure can be converted into new compounds with specific reactivation half-lives under pysiological conditions in order to modulate their pharmacokinetic properties to increase target selectivity, and to reduce toxic side-effects[5]. Heterobifunctional cross-linkers based on bicyclic carboxyamides substituted with appropriate coupling groups, are currently being constructed[6]. These cross-linkers should allow us to immobilize active components with defined stability on monoclonal antibodies or synthetic polymers in order to evaluate new principles of controlled drug release using a variety of anti-cancer agents.

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7. An unusually high pK_a-value of 6.9 was determined for a ß-carboxyamide[4].
8. Menger and Ladika[4] synthesized a β-carboxyamide which with a hydrolysis half-life of 7.5 min at a pD of 7.05 (corresponding to a pH of 6.6) at 21.5 °C. In contrast to amide **9a**, the β-carboxyamide exhibited a $T_{1/2(lim)}$ value of only about 3.5 min. Kirby and Lancaster[3] synthesized a maleic acid amide which showed a half-life of about 16 h at pH 6.6, but at pH 1 the lability of this carboxyamide increased to a half-life of a few seconds. Because of the architecture, the bicyclic carboxyamide 9**a** combines the unusual kinetic properties of the aforementioned different ß-carboxyamides and maleic amide derivatives. In contrast to the saturated compound **9a** the corresponding unsaturated compound revealed a very stable amide bond. We assume that the amide formation was accompanied by an intramolecular lactonization step. In this case the kinetically active carboxy group is protected. This compounds appear to be good candidates for liberation of the active caboxy group by an enzymatic reaction. Glüsenkamp, K.-H. and Rajewsky, M.F. unpublished results.
9. All different amides so far analysed showed basically the same structure-activity relationship. We synthesized a variety of labile amides mainly derived from amino-acid derivatives and bicyclic anhydrides; different characteristic labilities were found for a defined bicyclic ring-system: HNMe-CH_2- ≈ NH_2-CHR(COOH)- > NH_2-CH_2- > NH_2-CHR(COOMe) > NH_2-CHR(CONHR').
10. The program Hyperchem (Version 3.0, Autodesk; Munich, Germany) was used for calculation. Dewar, M.J.S., Zoebisch, E.G., Healy, E.F., Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, *107,* 3902-3909.
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