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Decomposition of 1-(ω-aminoalkanoyl)guanidines under alkaline conditions

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Abstract—The decomposition of some N^G-(ω-aminoalkanoyl)argininamides, which are key intermediates for the preparation of radiolabeled and fluorescent neuropeptide Y receptor ligands, prompted us to synthesize a small series of simple 1-(ω-aminoalkanoyl)guanidines, and to investigate these model compounds for stability in alkaline buffers. The degradation of acylguanidines was monitored by time resolved UV spectroscopy. The most labile compound, 1-(5-aminopentanoyl)guanidine, decomposed with a half life of 19 s to yield piperidin-2-one (pH 10.4 at 25 °C). In contrast the half life of 1-(6-aminohexanoyl)guanidine is 7.7 h, which is comparable to the hydrolysis of acetylguanidine ($t_{1/2} = 9.6$ h) in alkaline solution.

Guanidines are among the strongest non-anionic organic bases. The corresponding guanidinium ions are featuring the ability to form tight complexes with carboxylates, phosphonates, and sulfonates;[1] hence, the guanidine substructure plays an important role for molecular recognition processes in numerous biological and artificial systems. Numerous natural products and synthetic drugs comprising the guanidine motif are known.[2] As a natural amino acid arginine with its guanidino side chain is frequently found in the interaction sites of proteins.[3]

Due to their strong basicity ($pK_a \approx 12$ of the conjugate acids) guanidines are positively charged at physiological pH. This is a major disadvantage with respect to the pharmacokinetic profiles of guanidine derived drug candidates. Therefore, many efforts have been undertaken to find less polar bioisosteric replacements for the guanidine motif.[4–6]

By introduction of an electron withdrawing acyl substituent at the guanidino nitrogen the pK_a value can be reduced by 4–5 orders of magnitude.[7,8] Interestingly, N^G-acyl substituents do not necessarily impair the carboxylate binding properties of guanidines:[9,10] In our lab we discovered that the neuropeptide Y (NPY) Y_1 receptor antagonistic potency of the argininamide derivative BIBP 3226[11] can be enhanced by introduction of acyl substituents at the guanidine nitrogen.[8,12]

Based on this concept, we tried to synthesize N^G-(ω-aminoalkanoyl) substituted argininamides as precursors for radio- or fluorescence labeled NPY receptor ligands. However, it turned out that the ω-amino group can dramatically accelerate the decomposition of these compounds, depending on length and nature of the spacer. In this work we report on the degradation and the half lives of different ω-aminoalkanoylguanidines in alkaline buffer.

In an effort to label N^G-(5-aminopentanoyl)-substituted argininamides by acylation of the primary amino group in the presence of a base, almost exclusively the N^G-unsubstituted arginine derivative could be isolated. This supported the idea that the acyl-N^G bond was cleaved by intramolecular attack of the terminal amino function at the carbonyl C-atom. In order to test this hypothesis we synthesized 1-(5-aminopentanoyl)-guanidine (**1d**) as a model compound for investigations on stability.

Opportunely, acylguanidines in general exhibit strong UV absorbance around 230 nm.[13] This rendered

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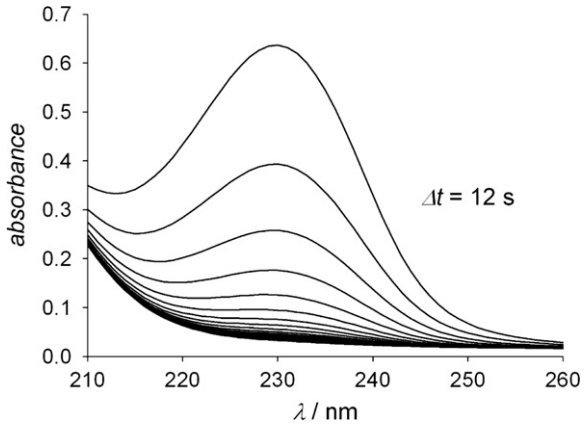


Figure 1. Time-dependent UV spectra of (5-aminopentanoyl)guanidine (**1d**) at pH 10.4.

possible to monitor the kinetics of the degradation of **1d** in alkaline buffers by time-resolved UV spectroscopy (cf. Fig. 1).

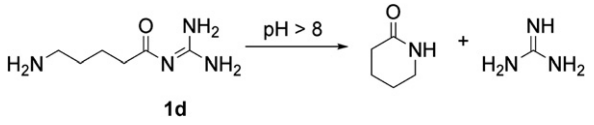
Remarkably, the absorption of the acylguanidine moiety was extinguished after only a few minutes. As acylguanidines in general are stable and isolable compounds, obviously, the presence of the terminal amino group dramatically enhanced the rate of decomposition by intramolecular nucleophilic attack (6-*exo*-trig ring closure). In fact piperidin-2-one (δ-lactam) was identified as the degradation product of 1-(5-aminopentanoyl)guanidine (**1d**) in alkaline solution by means of TLC and RP-HPLC (cf. Scheme 1).

The exponential decay of the acylguanidine absorbance at 230 nm allows for the calculation of (pseudo-)first order rate constants (k_{obs}) and the respective half-lives ($t_{1/2}$) by logarithmic plotting and linear regression of the data points.[†] For the degradation of 1-(5-aminopentanoyl)guanidine (**1d**) in alkaline aqueous buffers we observed half-lives of 18.4 min, 42 s, and 19 s at pH 8, 9, and 10.4, respectively.

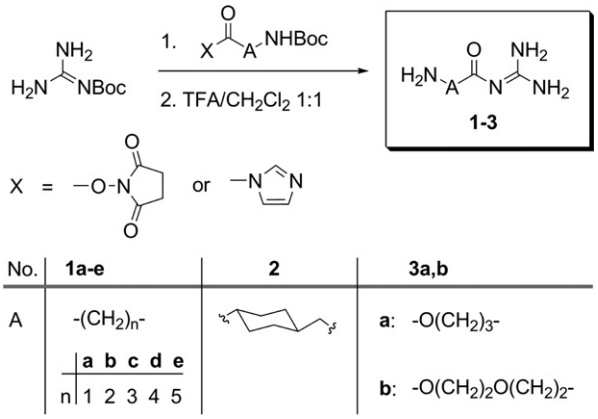
In quest of alternative, more stable spacers we tested various ω-aminoalkanoylguanidines of various chain lengths or with a cyclic scaffold for their stability in alkaline buffers. The compounds were prepared by acylation of 1-(*tert*-butoxycarbonyl)guanidine, which is accessible by treating guanidine hydrochloride with a hypostoichiometric amount of Boc_2O in 4 M NaOH.[14]

The *N*-*tert*-butoxycarbonyl (Boc) protected amino acids were either applied as succinimidyl esters, or activated with 1,1′-carbonyldiimidazole (CDI) and coupled with 1-Boc-guanidine. The resulting blocked aminoalkanoyl guanidines were deprotected with 50% trifluoroacetic acid (TFA) in CH_2Cl_2 (cf. Scheme 2). The obtained di-trifluoroacetates are sufficiently stable in acidic solutions.

[†] For details cf. Supplementary data.



Scheme 1. Degradation of 1-(5-aminopentanoyl)guanidine (**1d**) yielding δ-lactam and unsubstituted guanidine.



Scheme 2. Preparation of 1-(ω-aminoacyl)guanidines by acylation of 1-(*tert*-butoxycarbonyl)guanidine with activated *N*-Boc-protected ω-amino acid derivatives and subsequent removal of Boc-protecting groups with TFA-CH_2Cl_2 1:1.

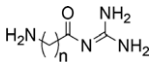
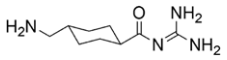
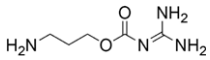
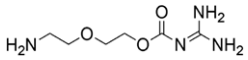
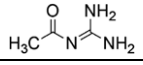
In addition, ω-aminoalkoxycarbonyl-guanidines, prepared from *N*-Boc protected amino alcohols, were probed as spacer variant. The alcohols were treated with disuccinimidyl carbonate (DSC) yielding the corresponding activated mixed carbonates, which were allowed to react with 1-Boc-guanidine (cf. Scheme 2).

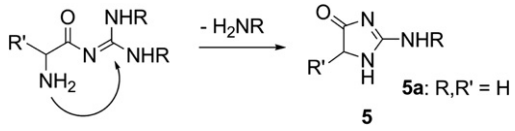
The degradation rate in alkaline borate buffer (pH 10.4) for all ω-aminoacylguanidines (**1**–**3**) was determined using time-resolved UV spectroscopy. Results are shown in Table 1. The slow reaction of acetylguanidine (entry **4**) and 4-(aminomethyl)cyclohexanecarbonylguanidine (**2**), which are unable to form lactams, reveals that there must be an alternative degradation pathway for acylguanidines—most probably hydrolytic cleavage.[13,15] Though, the isolation and characterization of the reaction products was beyond the scope of this study, some information about the potential degradation pathways can be derived from comparison of the reaction rates and the UV spectra.

In the case of glycylguanidine (**1a**) the formation of an α-lactam is implausible. Nevertheless, **1a** reacts much faster than acetylguanidine, indicating that the α-amino substituent participates in the degradation reaction. Rink et al.[16] found that N^{ω}-(α-Fmoc-aminoacyl)arginines form 2-amino-1*H*-imidazol-4(5*H*)-ones (**5**) when the Fmoc group is cleaved off in the presence of an excess of piperidine (cf. Scheme 3).

The UV spectrum of cyclic acylguanidine **5a** shows an absorption maximum at 225 nm (in phosphate buffer

Table 1. Degradation of various aminoacylguanidines in alkaline buffer (pH 10.4) at 25 °C; (pseudo-) first-order rate constants and half-lives

Compound	Structure	λ_{max}/nm	k_{obs}/min^{-1}	$t_{1/2}$
				
1a	$n = 1$	227	0.97	43 s
1b	$n = 2$	230	0.028	4.1 h
1c	$n = 3$	230	0.44	96 s
1d	$n = 4$	230	2.25	19 s
1e	$n = 5$	230	0.0015	7.7 h
2		231	0.0009	13 h
3a		215	0.0043	2.7 h
3b		215	0	∞
4		228	0.0012	9.6 h



Scheme 3. Intramolecular reaction of 1-(α-aminoacyl)guanidines according to Rink et al.[16]

at pH = 12)—but with a slightly lower molar extinction compared to acyclic acylguanidines.[15] These data are in good agreement with the slight hypsochromic shift (227 vs 224 nm) and the minor hypochromism we observed for the reaction of **1a** in alkaline buffer. Thus, the formation of **5a** is the most probable mechanism for the degradation of **1a**.

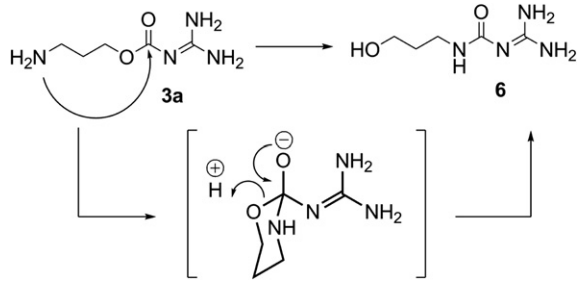
Within the series of linear 1-(ω-aminoalkanoyl) guanidines β-alanyl (**1b**) and 6-aminohexanoyl (**1e**) substituted guanidines exhibit the lowest degradation rates (cf. Table 1). The half lives for **1b** and **1e** are approximately 4 and 8 h, respectively. Obviously, the formation of corresponding β- or ε-lactams is significantly less favored than the formation of the δ-lactam, which proceeds within minutes.

For 1-(β-aminopropanoyl)guanidine (**1b**) the formation of a cyclic acylguanidine is less likely than in the case of **1a**. The expected absorption maximum at 233 nm, corresponding to 2-amino-5,6-dihydropyrimidin-4(1*H*)-one, could not be observed; moreover, the hypothetical product is known to be unstable in alkaline solution ($t_{1/2} = 4$ h at pH 12).[15] 1-(4-Aminobutanoyl)guanidine (**1c**), which can form a five-membered γ-lactam, decomposes at a comparably rapid rate as **1a** and **1d**. The most stable linear ω-aminoalkanoyl substituted guanidine in this series is **1e**, the decomposition of which proceeds only slightly faster than the alkaline hydrolysis of acetylguanidine (**4**).

The huge difference in the reaction rates of **1d**, the most labile compound, and **1e**, the most resistant compound is surprising since these aminoalkanoylguanidines differ only by one methylene group. Obviously, there is a substantial difference in the tendency to form six-membered and seven-membered lactams from 1-(ω-aminoalkanoyl)guanidines.

ω-Aminoalkoxycarbonyl substituted guanidines, which were taken into account as alternative spacer groups, are considerably more stable than the corresponding ω-aminoalkanoylguanidines. 1-Boc-guanidine and 1-[2-(2-aminoethoxy)ethyloxycarbonyl]guanidine (**3b**) were completely stable under the reaction conditions. The absorption maxima of the alkoxycarbonylguanidines are at shorter wavelengths (typically 215–225 nm) than those of alkanoylguanidines.

In contrast 1-(3-aminopropyloxycarbonyl)guanidine (**3a**), an oxa analog of the highly reactive aminoalkanoylguanidine **1d**, reacts with a half-life of 2.7 h. In principle the decomposition of both **3a** and **1d** can be facilitated in a similar way by intramolecular nucleophilic attack ('6-*exo*-trig') of the terminal amino group. However, the time-dependent absorption spectrum of **3a** in alkaline buffer shows the disappearance of the initial maximum at 215 nm and simultaneously the appearance of a new maximum at 224 nm. Since carbamates exhibit no absorbance in this region, the formation of a cyclic six-membered carbamate is implausible, whereas the preservation of the chromophoric acylguanidine substructure is more obvious. This can (only) be explained by a rearrangement of 1-(3-aminopropyloxycarbonyl)-guanidine (**3a**) to 1-(3-hydroxypropylaminocarbonyl)-guanidine (**6**) by initial nucleophilic attack of the terminal amino group at the acyl carbon atom and subsequent cleavage of the ester bond (cf. Scheme 4). Amidinourea shows a strong absorption maximum at approximately 220–225 nm,[17] which is in good agree-



Scheme 4. Hypothetical mechanism for the formation of putative isomerization product **6** from 1-(3-aminopropyloxycarbonyl)guanidine **3a**, supported by HPLC–MS analysis: after an incubation period of 60 min two peaks of identical mass, 161 Da (MH^+), with capacity factors of 0.55 (**3a**) and 1.88 (**6**) were detected, whereas at 0 min only one peak corresponding to **3a** was identified.

ment with the spectrum of the decomposition product of **3a**.

In conclusion, acylguanidines are promising, less polar bioisosteres of the strongly basic guanidino group. However, acylguanidines tend to decompose when subjected to alkaline conditions. The alkaline hydrolysis of acetylguanidine proceeds with a half-life of 9.6 h. Decomposition can be extremely accelerated if an intramolecular nucleophilic attack is possible, as demonstrated by the cleavage of 1-(5-aminopentanoyl)guanidine, which is completely converted to δ-lactam within minutes in alkaline solution. However, there are pronounced differences in the reaction rates of linear 1-(ω-aminoalkanoyl)guanidines depending on the length of the chain.

Compared to aminoalkanoylguanidines, the analogous aminoalkoxycarbonyl-substituted guanidines are considerably more stable toward alkaline hydrolysis. In general the oxa analogs are inert in aqueous buffer at pH 10.4 and 25 °C. However, the reaction can be facilitated by intramolecular nucleophilic groups in appropriate distance to the electrophilic center.

On one hand, the degradation pathways described in this study should be taken into account in the design and synthesis of aminoalkanoylguanidines such as N^G-substituted argininamides, which are useful building blocks for the preparation of fluorescent and radiolabeled NPY receptor antagonists. On the other hand, the 5-aminopentanoyl spacer could potentially serve as an easily cleavable linker for the immobilization of guanidines on solid support or as tunable protecting group for the guanidino function based on the principle of 'assisted cleavage'.

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Supplementary data

Supplementary data includes synthesis and analytical data of the discussed compounds as well as details about the kinetic measurements. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.07.147.

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