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Schiff bases derived from *p*-aminobenzyl alcohol as trigger groups for pH-dependent prodrug activation

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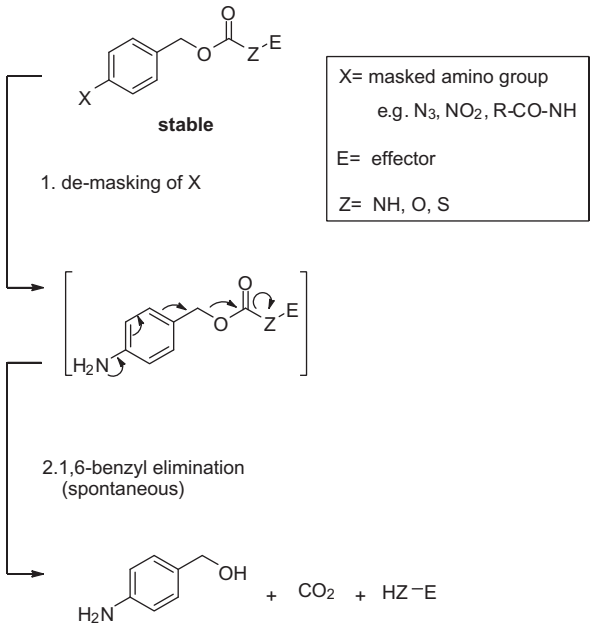
ABSTRACT

A number of novel acid-sensitive Schiff bases derived from *p*-aminobenzyl alcohol and various benzaldehyde derivatives were synthesized and were subsequently shown to trigger benzyl elimination reactions. The kinetics of acid-catalyzed hydrolysis at pH 5.0 as well as stability at pH 7.4 were studied using fluorogenic model compounds. Two fluoro-substituted Schiff bases showed efficient hydrolysis at pH 5.0 combined with a long-term stability at pH 7.4 and are considered suitable candidates for the development of anticancer prodrugs.

Coupling low-molecular weight cytotoxic drugs to suitable carriers (macromolecules, antibodies, or receptor-specific ligands) is a promising strategy to overcome the drawbacks associated with conventional anticancer chemotherapy.[1] For a controlled and site-specific release of the carrier-bound anticancer drug, the incorporation of acid-sensitive bonds (e.g., acyl hydrazone, acetal) between the drug and its carrier is an efficient and a versatile approach.[2] Since the cellular uptake of targeted prodrugs generally occurs via the endocytic pathway, these bonds are cleaved due to the significant shift from pH 7.2–7.4 in the blood or extracellular spaces to pH 4.0–6.5 in the various intracellular compartments such as endosomes or lysosomes. In the past, acyl hydrazone bonds have been successfully used for the development of acid-sensitive macromolecular prodrugs of doxorubicin.[3] Most drugs, however, do not provide an appropriate carbonyl or hydrazine moiety to form acyl hydrazone bonds. Therefore we set out to develop a more versatile method for a pH-specific prodrug activation that should be applicable to a large number of drugs. By incorporating a self-immolative linker, drugs with nucleophilic groups such as OH, NH_2, or SH can be chemically bound via transiently stable carbonate, carbamate, or *O*,*S*-thiocarbonate bonds. A pH-dependent triggering event causes a chemical breakdown of the linker with a subsequent release of the drug. This modular strategy comprising a trigger, a self-immolative linker, and the drug is often referred to as the double prodrug concept.[4]

A suitable self-immolative linker is the *p*-aminobenzyloxycarbonyl (PABC) system (Scheme 1) first described by Katzenellenbogen and co-workers[5] Upon activation, the PABC linker reacts in a



Scheme 1. The *p*-aminobenzyloxycarbonyl (PABC) system.

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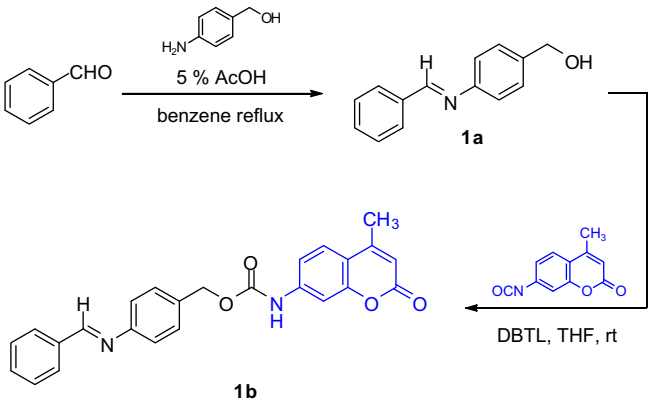
rapid and well-understood 1,6-benzyl elimination[6] and has therefore been employed for the design of various anticancer prodrugs[7] or even more complex self-immolative structures, such as oligomers,[8] polymers,[9] and dendrimers.[10] Self-immolation of the PABC linker is achieved by liberating the aromatic amino group from a masked precursor. In previous work, it was shown that the aniline of the PABC system could be effectively masked as an azide, a nitro group, or by acylation. De-masking was accomplished by reduction,[8,10] enzymatically[11] or chemically via the Staudinger reaction.[12]

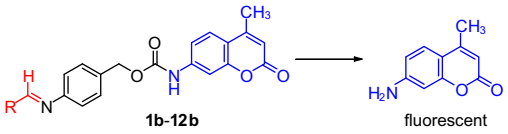


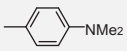
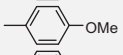
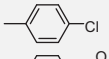
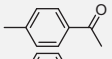
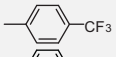
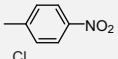
Scheme 2. Preparation of fluorogenic model compound **1b**.

Here we present novel PABC-based trigger groups that are activated by decreasing the pH. Since hydrolysis of Schiff bases (imines) occurs under mild acidic conditions, we assumed that this reaction would be suitable for de-masking the PABC's amino group. However, it had to be shown that the formation of a Schiff base offers an effective masking, i.e., prevents the PABC linker from benzyl elimination at neutral pH. As a model compound we synthesized the Schiff base derived from *p*-aminobenzyl alcohol and benzaldehyde that was subsequently reacted with 7-isocyanato-4-methyl-coumarin (Scheme 2).

The fluorophore 7-amino-4-methyl-coumarin (AMC) thereby serves as a model for an amino-functionalized drug. In contrast to the acylated form, free AMC shows a strong blue fluorescence (ex. 390 nm; em. 460 nm) and concentrations can be determined conveniently in the micromolar or even nanomolar range.[13] The release kinetics of the model compound (**1b**) was investigated at pH 5.0 (20 mM acetate buffer) and pH 7.4 (20 mM phosphate buffer) by measuring the increase in fluorescence that directly correlates with the liberation of AMC. All experiments were performed at low concentrations (10 μM) to minimize potential catalytic or inhibitory effects of the cleavage products.

Table 1
Yields of **1a**/**b**–**12a**/**b** and half-lives of model compounds **1b**–**12b** in buffer at pH 5.0 and 7.4 at 37 °C



Entry	R =	Yield **xa** (%)	Yield **xb** (%)	$t_{1/2}$ pH 5.0[a] (h)	$t_{1/2}$ pH 7.4[b] (h)
2a/**b**		77	87	0.28	26
3a/**b**		81	95	0.33	13
1a/**b**		77	66	0.57	8
4a/**b**		73	84	0.45	14
5a/**b**		71	76	0.62	36
6a/**b**		34	37	0.47	9
7a/**b**		90	85	1.02	36
8a/**b**		67	74	1.35	65
9a/**b**		36	74	0.83	50
10a/**b**		66	63	0.55	39
11a/**b**		75	82	0.57	89
12a/**b**		84	87	2.88	365

[a] Acetate buffer, 20 mM.
[b] Phosphate buffer, 20 mM.

At pH 5.0 we found a rapid release of AMC following a pseudo first-order kinetics with $t_{1/2}$ = 34 min. The hydrolysis of the Schiff base is the rate-determining step because the subsequent 1,6-benzyl elimination occurs rather rapidly ($t_{1/2}$ = 16 s at pH 5.0, see Supplementary data). In contrast, **1b** proved to be significantly more stable at pH 7.4 showing a half-life of 8 h. For the design of acid-sensitive anticancer prodrugs, however, a long-term stability at neutral pH ($t_{1/2}$ >48 h) is required. Thus we were interested in both which factors influence the kinetic behavior of **1b** and how the stability at neutral pH could be further improved.

To clarify these issues, we synthesized a small library of Schiff bases using benzaldehyde derivatives with different substituents (**2a**–**12a**). Reaction with 7-isocyanato-4-methylcoumarin according to Scheme 2 afforded the model compounds **2b**–**12b** of which the release kinetics were studied as described above (Table 1). We found that the imine bond is generally cleaved rapidly ($t_{1/2}$ = 17−173 min) at pH 5.0 whereas at pH 7.4 our model compounds proved to be significantly more stable ($t_{1/2}$ = 8−365 h). At both pH values we observed a marked influence of the substituents of the benzaldehyde component on the rate of cleavage with the general finding that electron-withdrawing groups have a stabilizing effect. In order to evaluate whether this effect can be described by Hammett's equation, we studied the linear free-energy relationships ($\log(k/k_0)$ vs σ_p) for the *para*-substituted compounds at both pH values (Fig. 1).

For pH 5.0, the plot shows a linear relationship in good approximation albeit with two exceptions: the data points for the substituents *p*-Ac and *p*-NMe_2. An explanation for the deviation of the dimethylamino substituent might be that this group is partially protonated at acidic pH whereas the σ_p value relates to the deprotonated form. However, we have no explanation for the significant deviation of the acetyl substituent. The reaction constant (ρ value) was calculated to be −0.57 thus indicating a moderate influence of substituents at pH 5.0. In contrast, for pH 7.4, a linear free-energy relationship was not found. The impact of substituents on the stability, however, was generally more pronounced than at pH 5.

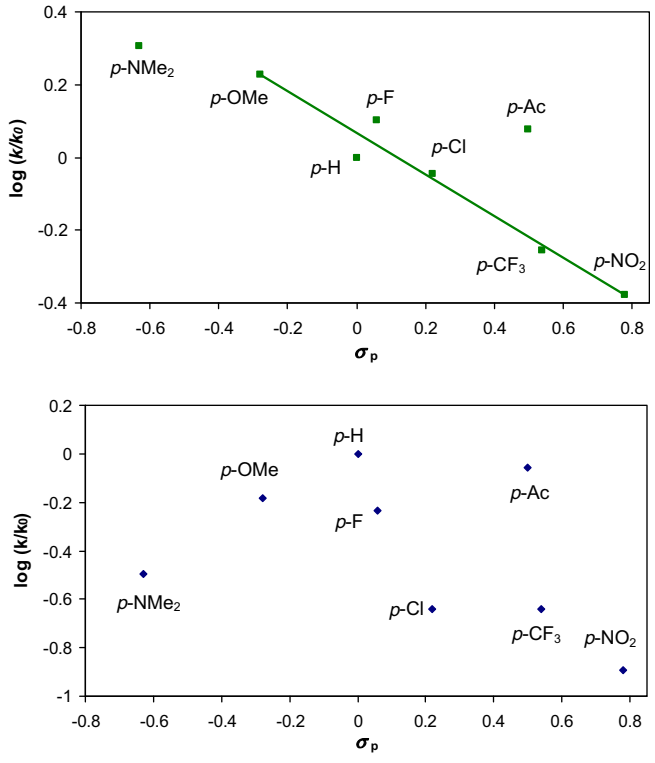


Figure 1. Linear free-energy relationships for the hydrolysis of *para*-substituted model compounds **1b**–**8b** at pH 5 (top) and pH 7.4 (bottom). σ_p values were obtained from the literature.[16]

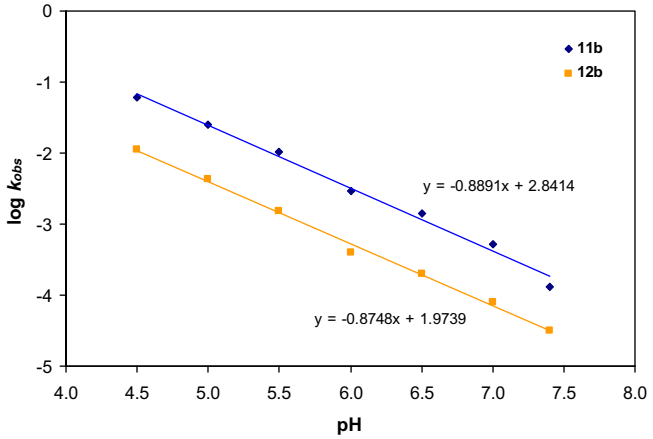


Figure 2. pH dependence of first-order rate constants for **11b** and **12b** (acetate buffer: pH 4.5–6, phosphate buffer: pH 6.5–7.4, 37 °C).

The stability of halogen-substituted Schiff bases at pH 7.4 could be further improved by introducing additional substituents as shown with the di-, tetra-, and pentafluoro- as well as the trichlorobenzaldehyde derivatives **9b**–**12b**. Especially the 2,3,5,6-tetrafluoro- and pentafluorobenzaldehyde derivatives (**11b**, **12b**) have proven to be considerably stable at neutral pH making them interesting candidates for medical applications. Therefore we studied the release behavior of both compounds more extensively over the physiologically relevant pH range (pH 4.5–7.4, Fig. 2).

Both compounds show a constant decrease in their rate of hydrolysis over the observed pH range. Between pH and log k_{obs} a linear relation was found in good approximation. This should be expected for a reaction with specific acid catalysis, that is, a reaction with an acid–base pre-equilibrium with only the protonated species (iminium) undergoing the next (rate-determining) reaction steps as shown for the hydrolysis of imines in previous mechanistic studies.[14] For a reaction with specific acid catalysis the first-order rate constant k_{obs} can be described as a function of pH: $k_{obs} = k_0 + k_{cat}\cdot[H^+]$ with k_0 as the rate constant for the uncatalyzed reaction. For $k_0 \ll k_{cat}\cdot[H^+]$ the slope of the graph should reach 1 in theory whereas for **11b** and **12b** a slope of ~0.9 was calculated. This means only a slight deviation from ideal acid catalysis. Moreover, the linear behavior suggests that the rate-determining step does not change within the examined pH range. Compounds **11b** and **12b** are the two model compounds with the most efficient pH response and were thus selected for the development of acid-sensitive prodrugs.

Although imine hydrolysis is a rather complex multistep reaction[15] and our analysis of the kinetic data does not take into account all effects that may result from general acid/base catalysis,[14] we feel that the general trend of pH-dependent stability is apparent within the spectrum of molecules that were studied.

In summary, we have developed a number of novel acid-sensitive Schiff bases that are capable of triggering benzyl elimination reactions. We showed that both acid sensitivity and stability at neutral pH can be adjusted and fine-tuned by varying the substituents of the benzaldehyde component. The Schiff base trigger groups, especially those derived from fluorine-substituted benzaldehydes, form the basis for developing prodrugs with anticancer drugs or self-immolative polymers that are degraded in an acidic environment.

Acknowledgment

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Supplementary data

Supplementary data (experimental section, characterization data, and ^{1}H and ^{13}C NMR spectra of all compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.06.055.

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