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

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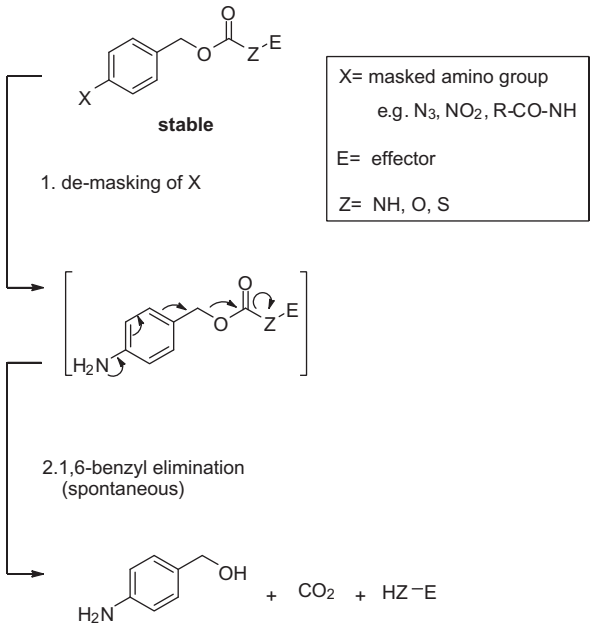
ABSTRACT

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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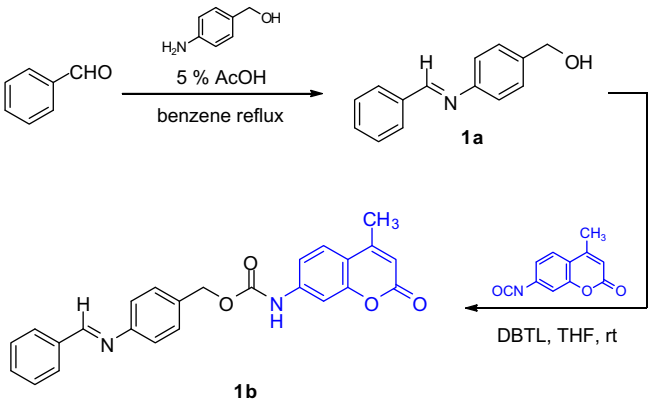
rapid and well-understood 1,6-benzyl elimination[6] and has therefore been employed for the design of various anticancer prodrugs[7] or even more complex self-immolative structures, such as oligomers,[8] polymers,[9] and dendrimers.[10] Self-immolation of the PABC linker is achieved by liberating the 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]

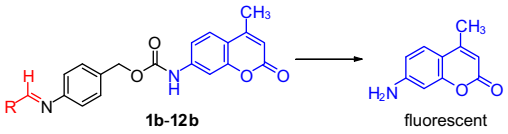


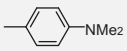
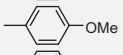
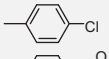
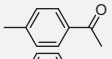
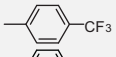
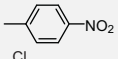
Scheme 2. Preparation of fluorogenic model compound **1b**.

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.