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Communications to the Editor

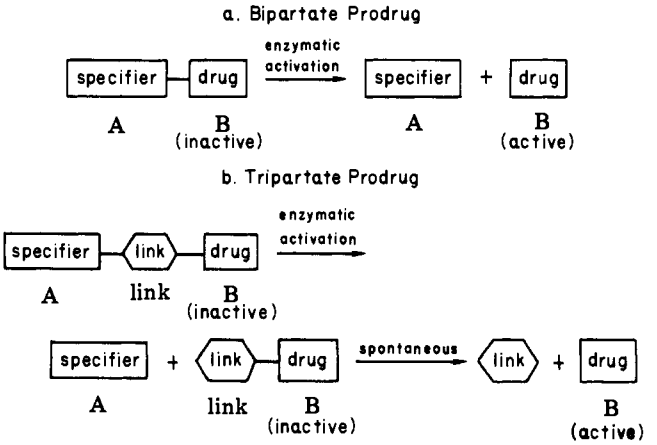
A Novel Connector Linkage Applicable in Prodrug Design

Sir:

We wish to report a chemical linkage that may be useful in solving certain problems in prodrug design.[1] Consider the bipartate prodrug illustrated in Scheme I consisting of a carrier or specifier moiety A and a drug moiety B. Moiety A may serve to target the drug to a particular site (e.g., by making it a specific substrate for a particular enzyme) or may confer other favorable properties on the prodrug; in vivo, enzymatic hydrolysis of the bond linking A to B releases active drug B in the body.[2] In certain cases, however, this basic prodrug strategy may fail. It is possible, for example, that the bipartate prodrug A–B may be unstable due to the inherent nature of the bond linking A to B; alternatively, the prodrug A–B may be quite stable, but the electronic or steric features of B may hinder hydrolysis of the A–B bond by the desired target enzyme. In either case, synthesis of a tripartate prodrug in which the specifier and drug are linked together by a special type of a connector group, A–link–B (Scheme I), may overcome these difficulties by spacing the drug B away from the specifier moiety A, so that the enzymatic activation now involves hydrolysis of the A–link bond rather than the A–B bond.[3] The link must be designed such that, following such activation, the remaining bond connecting the link and B spontaneously hydrolyzes under physiological conditions to release the active drug B.[4,5]

As a model of such a tripartate prodrug, we have synthesized [*p*-[N^{α}-[(*tert*-butyloxycarbonyl)lysyl]amido]-benzyloxycarbonyl]-*p*′-nitroanilide[6] (1, Scheme II) by reaction of *p*-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-[(trifluoroacetyl)lysyl]amido]benzyl alcohol [obtained by condensing N^{α}-Boc-N^{ϵ}-Tfa-Lys with *p*-aminobenzyl alcohol using *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline][7] with *p*-nitrophenyl isocyanate (prepared in situ from *p*-nitroaniline and carbonyldiimidazole in THF).[8] The trifluoroacetyl group was removed by treatment with tetramethylguanidine in acetonitrile–water (1:1) for 1 h at 25 °C.

Scheme I



In this model system (1), the N^{α}-Boc-Lys group is the specifier moiety A, the *p*-amidobenzyloxycarbonyl moiety is the link, and *p*-nitroaniline is the drug B. This tripartate prodrug is quite stable in aqueous buffers (0.05 M Bistris, pH 6.9) in the absence of trypsin ($t_{1/2} \approx 40$ h at 25 °C), but in the presence of trypsin (2.5 μg/mL) undergoes rapid hydrolysis to release the chromophore *p*-nitroaniline **5** ($t_{1/2}$ after activation ≈ 11 min at 25 °C).[9] The rapid release of *p*-nitroaniline following activation is presumably due to the conversion of the weakly electron-donating acylamido group (σ_p^+ –0.6) to the strongly electron-donating

(1) For general reviews of prodrugs, see (a) Sinkula, A. A.; Yalkowsky, S. H. *J. Pharm. Sci.* **1975**, *64*, 181. (b) Higuchi, T.; Stella, V., Eds. *ACS Symp. Ser.* **1975**, *no. 14*. (c) Stella, V. J.; Himmelstein, K. J. *J. Med. Chem.* **1980**, *23*, 1275.

(2) For an example illustrating that certain peptidyl derivatives of antitumor agents are hydrolyzed selectively by transformed cells in culture, see: Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A.; Weber, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2224.

(3) We have encountered prodrug instability and inadequate substrate activity in connection with our work on peptidyl derivatives of antitumor agents (cf. ref 2), and we have found that the linker described in this paper is useful in overcoming these problems (P. L. Carl, P. K. Chakravarty, M. J. Weber, and J. A. Katzenellenbogen, in preparation).

(4) Two prodrug linkages that involve a sequences cascade of hydrolytic steps have been described: the spontaneous cyclization of a phthalide derivative (Cain, B. F. *J. Org. Chem.* **1978**, *41*, 2029) and the elimination of formaldehyde upon hydrolysis of acyloxymethyl esters (reviewed by Sinkula, A. A., in ref 1b, Chapter 2).

(5) We would like to propose the term "self-immolative connector" to describe a connector linkage of this type.

(6) Abbreviations used: Boc, *tert*-butyloxycarbonyl; Tfa, trifluoroacetyl.

(7) Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651.

(8) Stabb, H. *Angew. Chem., Intl. Ed. Engl.* **1962**, *1*, 351.

(9) The rate-limiting step of hydrolysis of 1 in the presence of this concentration of trypsin (2.5 μg/mL) is hydrolysis of the lysyl bond to the aminophenyl group. Increasing concentrations of trypsin (up to 100 μg/mL) give more rapid rates of appearance of *p*-nitroaniline, but even at the highest trypsin concentrations there is no evidence of a lag in the production of *p*-nitroaniline. Therefore, rate of solvolysis of the *p*-aminobenzyl carbamate group is still fast relative to the rates of enzymatic cleavage of the lysyl group attainable with trypsin concentrations up to 100 μg/mL. Assuming a ρ value of –4.5 (cf. ref 10), we would predict that the half-life of the *p*-aminobenzyl carbamate to be ca. 1.5 min.

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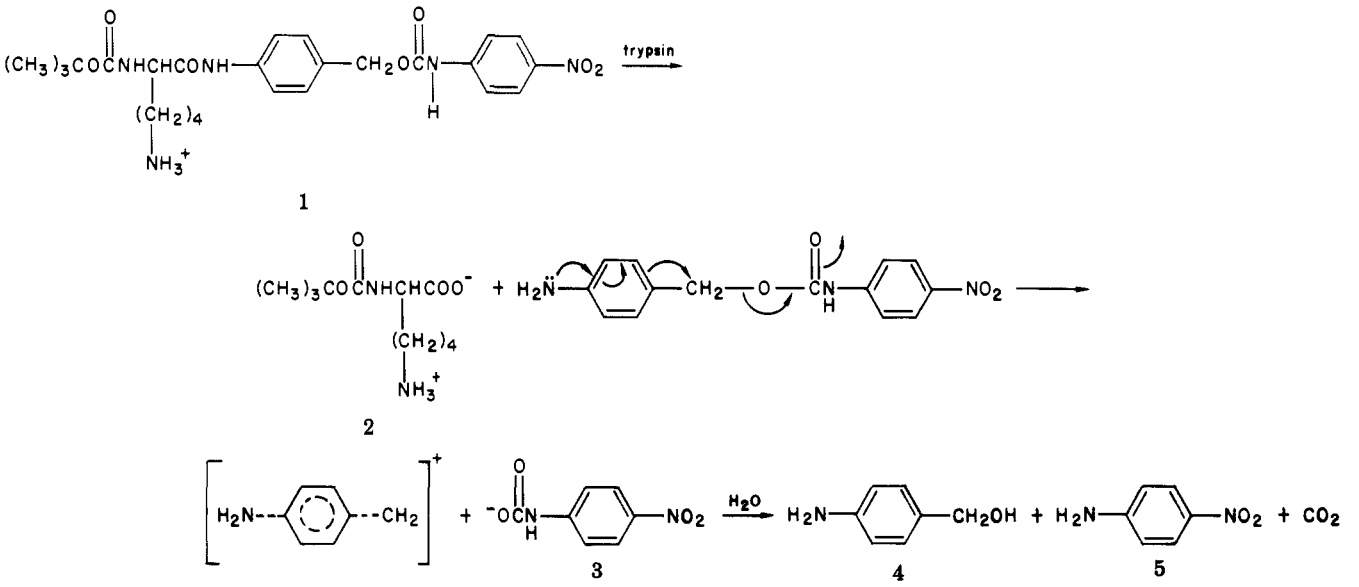
Scheme II



amine group (σ_p^+ −1.31). This additional release of electron density into the π system, which would stabilize the development of positive charge on the benzylic carbon atom, greatly labilizes this system toward solvolytic cleavage of the benzyl–carbamate bond.[10-12] The carbamic acid that is released (3) is unstable and rapidly loses CO_2 to yield *p*-nitroaniline (5). The chromophore can be detected by colorimetry or thin-layer chromatography, the latter procedure also revealing the presence of the expected fragments N^{α}-Boc-Lys (2) and *p*-aminobenzyl alcohol (4). Trypsin-catalyzed release of *p*-nitroaniline from 1 is dependent on a free lysine side chain, since *p*-nitroaniline is not released upon treatment of *p*-[(N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-[(trifluoroacetyl)lysyl]amido]benzyl-*p*′-nitroanilide (N^{ϵ}-Tfa-1) with trypsin. This latter experiment also demonstrates that the carbamate group itself is not susceptible to trypsin hydrolysis. Trypsin hydrolysis of 1 is also inhibited by the trypsin inhibitor N^{α}-tosyl-L-lysyl chloromomethyl ketone. Chymotrypsin has no effect on 1.

In this report we have demonstrated that it is possible to prepare a model of a prodrug in which the connector between a specifier moiety and a drug is released by a sequence of hydrolytic steps, the first involving an enzymatic cleavage and the second involving a solvolysis that proceeds spontaneously, but only after the first step occurs. These results have been demonstrated so far only in an in vitro system, and while it is possible that further complications may develop in the use of such agents in vivo,[13] the successful demonstration of the principle upon which this connector was designed provides the medicinal chemist with greater flexibility in approaching the design of prodrugs. In future publications, we will report on the application of this type of prodrug linkage to problems we have encountered in the synthesis of peptidyl derivatives of cytosine arabinoside and adriamycin that we are preparing as protease-activated antitumor prodrugs.[2,3]

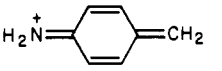
Acknowledgment. This work was supported by Grant CA 23498 from the National Cancer Institute.

(10) For S_N1 solvolysis of electron-rich benzylic derivatives, ρ values in the range of −4.4 to 4.8 have been reported: Brown, H. C.; Okamoto, Y. *J. Am. Chem. Soc.* **1958**, *80*, 4979. Therefore, one would expect the solvolytic lability of the benzyl carbamate to be increased ca. 1600-fold by deacylation of the *p*-amino group.

(11) Related labilizations of benzylic carbamate bonds have been reported: Kemp, D. S.; Hoyng, C. F. *Tetrahedron Lett.* **1975**, 4625. Teicher, B. A.; Sartorelli, A. C. *J. Med. Chem.* **1980**, *23*, 955.

(12) While the example described here involves the release of a weakly basic amine *p*-nitroaniline, the solvolytic lability of the benzylic carbamate should be relatively unaffected by changes in the nature of the amine-containing drug moiety, because it is quite effectively insulated electronically. Furthermore, in preliminary experiments, we have found that the *p*-[N^{α}-[(*tert*-butyloxycarbonyl)lysyl]amido]benzyloxycarbonyl derivatives of aniline and of cytosine arabinoside (at N^4) also release the amine component rapidly upon treatment with trypsin (P. K. Chakravarty and P. L. Carl, unpublished).

(13) A reviewer has pointed out that solvolysis of the *p*-aminobenzyl carbamate might produce the iminoquinone methide (I). This intermediate might be reactive toward cellular nucleophiles (e.g., glutathione) and at high concentrations might effect some toxicity. The carbamate linkage may also be susceptible to enzymatic hydrolysis in vivo. we have demonstrated in this study, however, that the carbamate is inert to both trypsin and chymotrypsin.



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(14) Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27514.

Philip L. Carl,[14] Prasun K. Chakravarty
John A. Katzenellenbogen*
Department of Chemistry
University of Illinois
Urbana, Illinois 61801

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