Remote Enzyme-Coupled Amine Release

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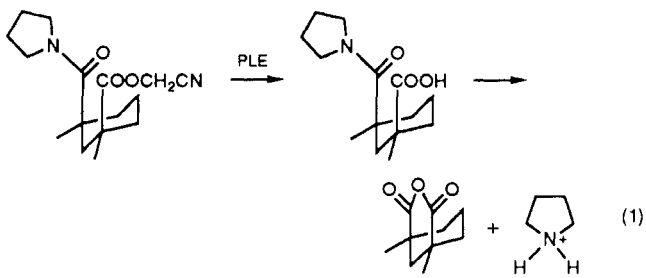
Received February 27, 1990

Summary: A remote enzyme-coupled amine release system has been developed in which biochemical and chemical "demasking" processes are sequentially linked via an enforced 1,3-diaxial disposition. Thus, pig liver esterase hydrolyzes an ester to an acid, and the acid promotes a fast intramolecular cleavage of an amide to an amine. The sequence has potential pharmacokinetic relevance because it allows enzyme-specific discharge of amine-based drugs following their trans-membrane journey as uncharged amides.

Consider Scheme I in which the central idea motivating our experiments is expressed. A masked drug is converted into an active form with the aid of a catalyst. The catalyst, itself present initially as an inert derivative, becomes activated by an enzyme. If the masked drug and the masked catalyst are attached side-by-side to the same molecular framework, then the active drug can be released rapidly and specifically upon exposure to the enzyme. We call such a combination of events a "remote enzyme-coupled drug release". The concept, although not precisely expressed in these terms, has been recently exploited by Norbeck et al.[1] and Johnson et al.[2]

Remote enzyme-coupled drug release systems have a notable advantage over conventional prodrugs.[3] If a prodrug consists, for example, of an amine masked as an amide, then an enzyme will generate the drug only when an enzyme is capable of hydrolyzing the amide. This may occur much more efficiently with one particular drug than with another according to the specificity of the enzyme. But with the remote system, a wide variety of drugs may be used as long as there exists an enzyme capable of deprotecting the nearby catalyst. The uniqueness of the approach derives from the fact that drug release depends on two distinct stages: a biochemical step not directly involving the drug moiety, and a chemical step in which the drug is liberated.

We have developed a remote release system based on a recently discovered and remarkably fast amide hydrolysis occurring when an amide and a carboxyl exist in a 1,3-diaxial arrangement.[4] As a result of spatiotemporal[5] and compressive factors,[6] such an amide hydrolyzes at pH 7 and 21 °C in minutes (constituting a $>10^{12}$ catalysis by the carboxyl group). The possibility existed, therefore, of masking the acid as an ester, freeing the carboxyl with pig liver esterase (PLE), and observing a subsequent amide cleavage with release of the amine (eq 1). The sequence has potential pharmacokinetic relevance because it allows enzyme-specific discharge of amine-based drugs following their trans-membrane journey as uncharged amides.



(1)

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Scheme I

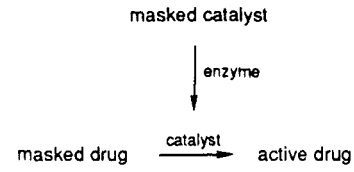

Table I. Yields of Amine Production in Eq 1 with and without Pig Liver Esterase (PLE) and Chymotrypsin (Chy)

enzyme	pD	time, h	yield, %
none	8.2	312	<1
PLE[a]	8.2	39	75
PLE	8.2	83	95
PLE	7.4	50	95
Chy[b]	7.4	266	2

[a] See text for experimental conditions. [b] α-Chymotrypsin (Type II, 114 units) was added to ester–amide (1.0 mg) dissolved in a pD 7.40 phosphate buffer. The progress of the reaction at 21 °C was monitored by NMR as described for PLE.

The ester–amide in eq 1 was prepared by (a) catalytic hydrogenation of benzene-1,3-dicarboxylic acid over Rh/C; (b) double esterification with $SOCl_2$ and methanol; (c) double methylation with LDA and dimethyl sulfate to give the dimethyl ester of *cis*-1,3-dimethylcyclohexane-1,3-dicarboxylic acid; (d) saponification of the esters; (e) formation of the bicyclic anhydride using Ac_2O; and (f) addition first of pyrrolidine/triethylamine and then of chloroacetonitrile to the anhydride in DMF. Ester–amide, formed in 51% overall yield, was characterized fully.[7]

The rate of the PLE-catalyzed ester cleavage in eq 1 was measured by continuous titration of the resulting acid using a pH-stat. In a typical experiment, ester–amide (8.0 mg, 27 μmol) in 6.0 mL of borate buffer (1.1 mM, D_2O) was mixed with PLE (Sigma E3128, 1144 units); subsequent ester hydrolysis was monitored at pD 8.0 or 8.4 and 21 °C. Half-lives under these conditions were 12.3 and 10.4 h, respectively. The rate of amine release was obtained from the appearance of the pyrrolidine NMR signal at δ 3.22 ppm. Typical conditions included ester–amide (1.0 mg, 3.4 μmol), 0.75 mL of phosphate buffer (0.11 M, D_2O), and PLE (143 units) at pD 7.4 and 21 °C. Acid–amide, being too reactive to isolate, was prepared in situ from the anhydride; spontaneous reformation of anhydride (second

(1) Norbeck, D. W.; Rosenbrook, W.; Kramer, J. B.; Grampovnik, D. J.; Lartey, P. A. *J. Med. Chem.* **1989**, *32*, 625.
(2) Johnson, C. D.; Lane, S.; Edwards, P. N.; Taylor, P. J. *J. Org. Chem.* **1988**, *53*, 5130.
(3) Amidon, G. L.; Pearlman, R. S.; Leesman, G. D. In *Design of Biopharmaceutical Properties through Prodrugs and Analogs*; Roche, E. B., Ed.; Acad. Pharmaceutical Sci.: Washington, 1977; pp 281–315.
(4) Menger, F. M.; Ladika, M. *J. Am. Chem. Soc.* **1988**, *110*, 6794.
(5) Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128.
(6) Spatiotemporal acceleration is obviously far more important than steric compression because the acid–amide in eq 1 is free to flip into the 1,3-diaxial methyl conformation were it prone to do so. Since the resulting methyl/methyl interaction is worth 3.7 kcal/mol according to Allinger[8] and repulsion between the acid and amide cannot exceed this, relief of compression cannot contribute more than 10^3 to the fast intramolecular cyclization.
(7) Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58. Found: C, 65.64; H, 8.28; N, 9.60. HRMS: Calcd for $C_{16}H_{24}N_2O_3$ 292.1787. Found: 292.1785. Spectroscopic data (IR and NMR) are consistent with the structure; mp 65–66 °C.
(8) Allinger, N. L.; Miller, M. A. *J. Am. Chem. Soc.* **1961**, *83*, 2145.

0022-3263/90/1955-3006$02.50/0

step in eq 1) was also followed via pyrrolidine formation in the NMR.

As seen in Table I, no ester hydrolysis was observed after 312 h at pD 8.2 in the absence of pig liver esterase. On the other hand, under the NMR conditions mentioned above, addition of the enzyme gave ester hydrolysis in a >95% yield after 50 h at pD 7.4. The rate of amine release following this ester hydrolysis depended strongly upon the pD. For example, amide cleavage (i.e. the second step in eq 1) has half-lives of 10.5 min at pD 6.50, 19 min at pD 6.90, and 2.7 h at pD 7.40. Intramolecular acid-catalyzed amide hydrolysis clearly diminishes in rate as the carboxyl ionizes.[4] Overall generation of amine has an optimal half-life of about 12 h near pD 7.4. Below that pD, the PLE-catalyzed esterolysis begins to slacken; above pD 7.4, amide cleavage becomes rate-determining under our standard conditions.[9]

(9) The kinetic behavior is similar to that described for a Kemp's triacid derivative detailed in ref 4 except that the cross-over point for the rate-determining step is shifted somewhat to more basic pH values. The cyclohexyl byproduct (visible in the NMR) is either the anhydride at lower pH's or the dicarboxylate at higher pH's.

When a primary amine, tryptamine, was used instead of pyrrolidine, the same mechanism applied although the overall rate of amine cleavage was an order of magnitude less. Obviously, primary amine release is too slow for a workable drug therapy, a problem that could, possibly, be rectified via a more reactive ester functionality. Structural optimization has not been attempted, so that at this stage we can only present the general concept and offer a prototypical example. One encouraging point with regard to potential practicality, however, relates to the chymotrypsin data in Table I. Since chymotrypsin, a pancreatic protease, was found incapable of catalyzing ester or amide hydrolysis in our compounds, a drug masked as in eq 1 could likely survive passage through the intestinal tract.

In summary, we have developed a new type of prodrug in which biochemical and chemical "demasking" processes are sequentially linked via an enforced 1,3-diaxial disposition.

Acknowledgment. This work was supported by the National Institutes of Health and the National Science Foundation.

Reverse Cope Eliminations. Pyrrolidine and Piperidine *N*-Oxides by Intramolecular Addition of N,N-Disubstituted Hydroxylamines to Unactivated Double Bonds

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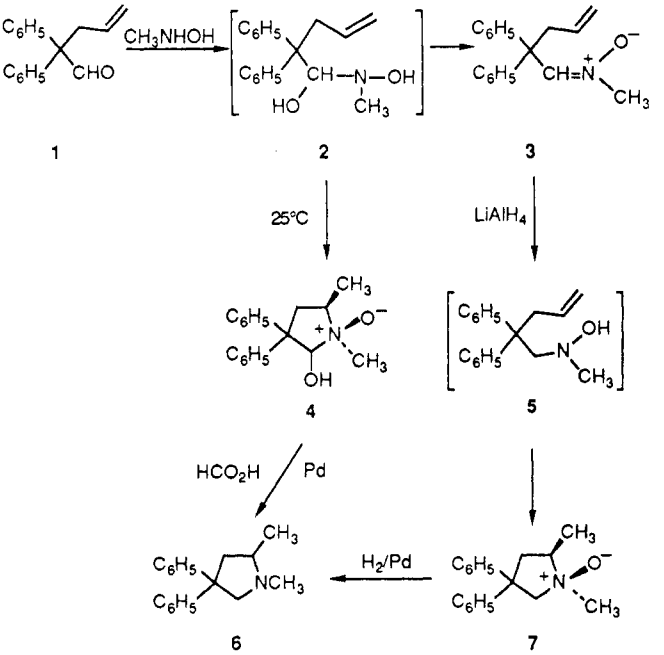
Received March 15, 1990

Summary: *N*-(4-Pentenyl)- and *N*-(5-hexenyl)-*N*-methylhydroxylamine and some of their derivatives cyclize under mild conditions in a concerted reverse Cope elimination to give 2-alkylpyrrolidine and 2-alkylpiperidine *N*-oxides.

In 1976, House and co-workers reported the cyclization of substituted *N*-(4-pentenyl)- and *N*-(5-hexenyl)-hydroxylamines to 1-hydroxy-2-methylpyrrolidines and 1-hydroxy-2-methylpiperidines, respectively,[1-3] and suggested a radical-chain mechanism for this reaction. We report here some preliminary observations on the scope and mechanism of the analogous transformation of unsaturated N,N-disubstituted hydroxylamines into cyclic *N*-oxides.[4]

When 2,2-diphenyl-4-pentenal (1) was allowed to react with *N*-methylhydroxylamine in ethanol at room temperature, the desired nitrone **3** was obtained in only 45% yield (Scheme I). The second product, formed in 51% yield, was shown to be 1,5-dimethyl-3,3-diphenyl-2-pyrrolidinol 1-oxide (**4**) by elemental analysis, ^{1}H NMR spectroscopy, and reduction to 1,2-dimethyl-4,4-diphenylpyrrolidine (**6**). *N*-Oxide **4** is the product of a formal reverse Cope elimination[5] of intermediate **2**. Reduction

Scheme I



(1) House, H. O.; Manning, D. T.; Melillo, D. G.; Lee, L. F.; Haynes, O. R.; Wilkes, B. E. *J. Org. Chem.* **1976**, *41*, 855. House, H. O.; Lee, L. F. *J. Org. Chem.* **1976**, *41*, 863.

(2) The reaction was independently discovered by Oppolzer, W.; Siles, S.; Snowden, R.; Bakker, B. H.; Petrzilka, M. *Tetrahedron Lett.* **1979**, 4391, footnote 5.

(3) For additional examples of this cyclization, see: (a) Black, D. St. C.; Doyle, J. E. *Aust. J. Chem.* **1978**, *31*, 2317. (b) Yamada, F.; Makita, Y.; Suzuki, T.; Somei, M. *Chem. Pharm. Bull.* **1985**, *33*, 2162. (c) Yamada, F.; Hasegawa, T.; Wakita, M.; Sugiyama, M.; Somei, M. *Heterocycles* **1986**, *24*, 1223. (d) Lamanec, T. R.; Bender, D. R.; DeMarco, A. M.; Karady, S.; Reamer, R. A.; Weinstock, L. M. *J. Org. Chem.* **1988**, *53*, 1768 and references cited there. Karady, S.; Corley, E. G.; Abramson, N. L.; Weinstock, L. M. *Tetrahedron Lett.* **1989**, *30*, 2191. (e) Carling, R. W.; Leeson, P. D. *Tetrahedron Lett.* **1988**, *29*, 6985. (f) Leeson, P. D.; James, K.; Baker, R. *J. Chem. Soc., Chem. Commun.* **1989**, 433.

(4) For a transformation in which one of the steps was proposed to involve a reverse Cope elimination of an N,N-disubstituted hydroxylamine, see: Takahashi, S.; Kusumi, T.; Sato, Y.; Inouye, Y.; Kakisawa, H. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1777.

(5) For a review of the Cope elimination, see: Cope, A. C.; Trumbull, E. R. *Org. React.* **1960**, *11*, 317.

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