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Study into the cyclization of an epoxy-alcohol to the energetically disfavored product by peptides from non-biased combinatorial libraries

Gabriela Chiosis *

Department of Chemistry, Columbia University, 3000 Broadway, New York, NY 10027, USA

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Abstract

Catalysis of a reaction to products via an energetically disfavored pathway to be performed by small molecules is not documented to date. To question that, we chose to study the possible cyclization of an epoxy–alcohol to the product interdicted by Baldwin's rules. For achieving this goal, thousands of randomly chosen peptidic combinatorial library members were screened against a transition state analog of the disfavored reaction. This method led to the discovery of several sequences which could behave as catalysts of the reaction. Complexes between the epoxy–alcohol and certain peptides were studied using molecular modeling to shed light on the binding and mode of action of these peptides and the reason they were selectively chosen. Certain re-synthesized peptides amounted to a 10–15 fold increase in the production of the disfavored product when added to the reaction.

Keywords: catalysis; transition states; combinatorial chemistry; molecular modeling/mechanics.

1. Introduction

Energy barriers encountered by reactants along the reaction coordinates dictate the distribution of products in the kinetically controlled[1] processes. Once a barrier is lowered, an important change in the distribution could be achieved. Such shift could be accomplished by a molecule that binds the transition state intermediate of the energetically disfavored reaction lowering the activation energy and, therefore, catalyzing the formation of that product. An example of kinetically controlled process is the intramolecular cyclization in which formation of products is dictated by stereoelectronic constraints in the transition state. Lerner and co-workers[2] studying the cyclization of an epoxy-alcohol, have shown that

* Requests should be addressed to: Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., Box 271, New York, NY 10021, USA. Tel: (212) 639-8929; fax: (212) 717-3627; e-mail: chiosisg@mskmail.mskcc.org (G. Chiosis)

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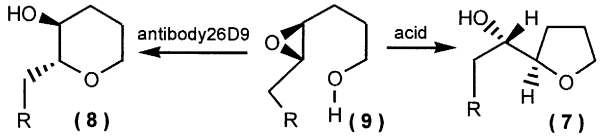
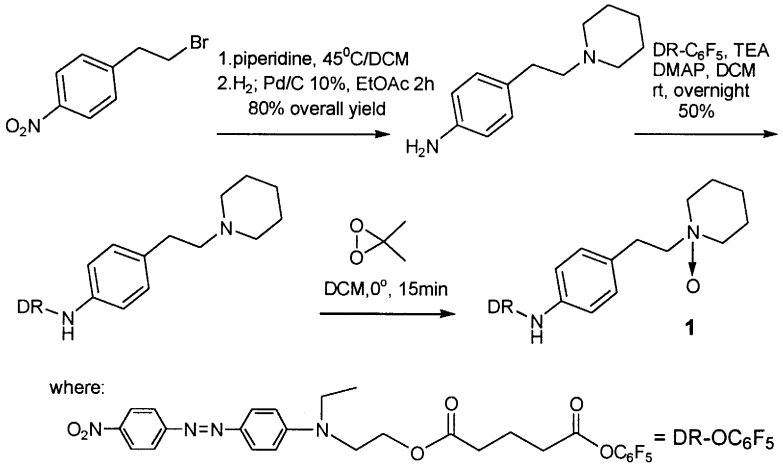
an antibody raised against a transition state analog of the disfavored reaction led predominantly to the product of that reaction (**8**) (Scheme 1). In the presence of an acid the epoxy-alcohol **9** leads exclusively to the formation of **7**, a 5-membered ring derivative.[3] However, the structure of the induced antibody, and therefore of the binding site, was not determined nor is there data in the literature about a small molecule that could perform such task. Therefore, we set out to screen non-biased combinatorial libraries against a similar transition state analog in the hope of finding a simple molecule of known structure that could catalyze the formation of the 6-membered ring derivative, **8**.



Scheme 1. Distribution of products in the reaction catalyzed by antibody 26D9 and by acid

2. Results and discussion

The transition state analog used was an *N*-oxide, an attractive candidate for the induction of a catalyst.[2] The cationic nitrogen was expected to generate amino acid residues that would stabilize the carbocation appearing along the reaction, while the anionic oxygen was expected to induce positively charged amino acids to assist the epoxide ring opening. To facilitate the identification of compounds on solid support that bound the transition state analog, we labeled it with a red dye.[4] Disperse Red 1, an azo dye with high absorption at 500 nm was selected for attachment to the *N*-oxide through a linker, resulting in the substrate **1** (Scheme 2).†



Scheme 2. Synthesis of the transition state analog **1**

The combinatorial libraries used[5] (Fig. 1) were randomly selected. Both are peptide libraries synthesized on polystyrene; however, they contain different amino acids linked to the bead through a scaffold, as in the case of library GLPro or simply a linker, as for library SSY. The assays were carried out in chloroform.[6] The beads were continuously shaken in the solution and the concentration in **1** was

† Abbreviations to text: DMAP, dimethylaminopyridine; Aa, amino acid; DMDO, dimethyldioxirane; TEA, triethylamine; MCMM, Monte Carlo multiple minimum; LMCS, low mode conformational search; GB/SA, generalized Born/surface area.

lowered each day by addition of solvent until the solution became colorless but some beads retained a red coloration. Equilibration was obtained under these conditions after 6–8 days. If beads were picked only after one day of shaking, all decoded beads showed a high abundance in Asp and Glu, revealing unspecific polar interactions between the carboxylates and the *N*-oxide (Fig. 2). After a few days, however, different sequences started appearing, all containing Lys and Pro. The analysis of results from screenings clearly revealed that Lys was present adjacent to a Pro in all the active beads. In the library GLPro, 70% of the active beads carried Pro in the position A1 and Lys in A3 (Fig. 2). Patterns were found several times in the screening results for library SSY. Similar to the library GLPro, the results disclosed the presence of a Lys neighboring one or two Pros (Table 1).

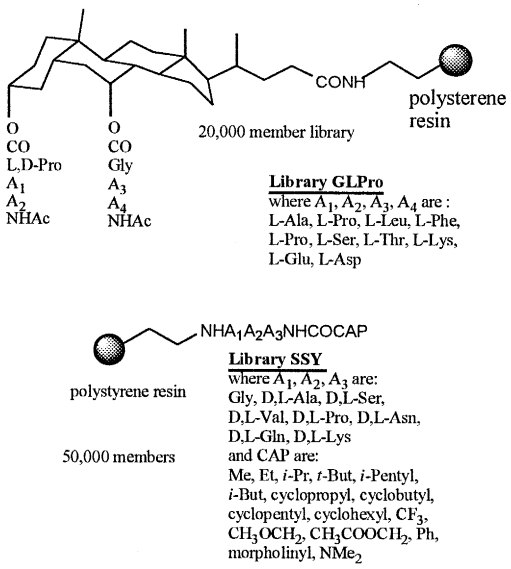


Fig. 1. Combinatorial libraries screened against substrate **1**

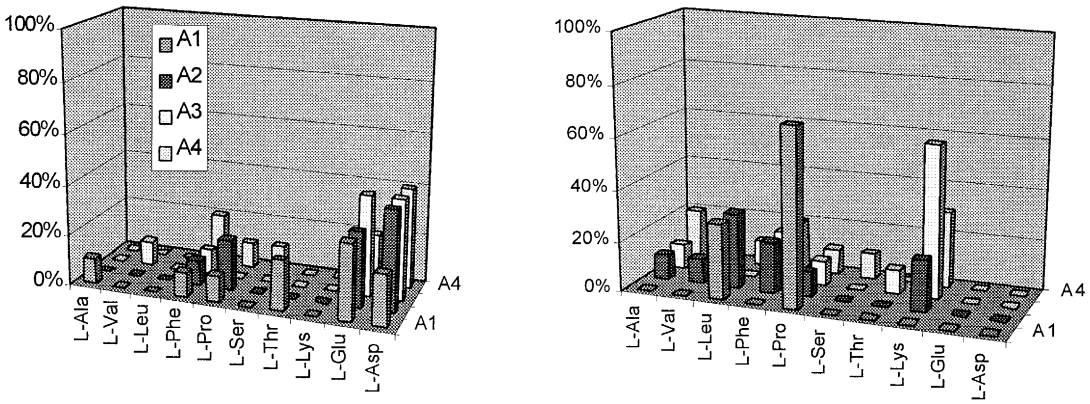


Fig. 2. Results from the assay of library GLPro. Red beads picked after one day of equilibration (first graph) and six days (second graph)

To understand why these sequences were selected and how they work, we decided to study some peptides using molecular modeling. Several sequences found from the screening of library SSY against the *N*-oxide **1**, were chosen to be studied. Conformational searches on the complexes of these peptides with the epoxy-alcohol were successful using MCMM and LMCS methods alternatively.[7] Simulations

Table 1
Sequences from library SSY showing binding activity for **1**

A_1	A_2	A_3	CAP
LLys	LPro	DPro	Variable
LPro	DLys	DPro	Variable
DPro	DLys	LPro	Variable
LLys	DPro	DPro	Variable
LPro	LLys	LPro	Variable
LAa	LPro	DLys	Variable
LAa	DLys	LPro	Variable
LLys	LPro	DAa	Variable
LLys	LAa	LPro	Variable
DLys	DPro	LAa	Variable
LLys	Variable	LPro	Variable
DLys	Variable	DPro	Variable

were run using the GB/SA solvation method for chloroform and AMBER* force field, as implemented in version 6.0 Macromodel. The results (as pictured for peptide **3**) show conformations in which the NH_3^+ of the Lys can be positioned for a 6-endo epoxide opening (Fig. 3). This is the outcome of a bent structure of the peptides favored by the conformationally restrictive Pro and by internal hydrogen bonds. The positioning of the NH_3^+ was induced by the location of the negatively charged oxygen of the *N*-oxide. These structures suggest that the hydroxyl could be favorably positioned for attacking the carbocation generated after the epoxide opening by Lys. Encouraged by these results we decided to re-synthesize some of these sequences and check if their addition to the epoxy-alcohol increases the amount of the formed disfavored product.

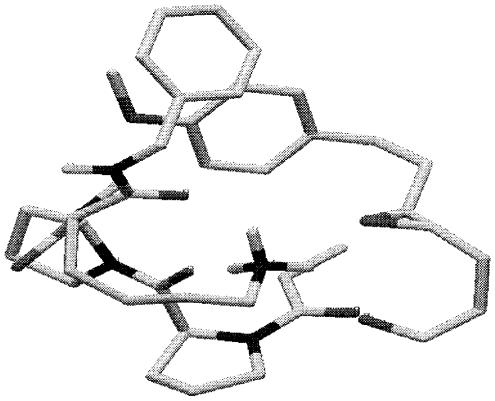


Fig. 3. Complex of peptide **3** with epoxy-alcohol **9**

The peptides **2**, **3**, **4**, **5** and **10** (Fig. 4) were synthesized in solution using standard peptide methodology.[8] Additionally, protection of the OH of the epoxy-alcohol with methoxydimethyl (MDM) was performed to give the substrate **6**.[9] This was necessary due to the high instability of the epoxy-alcohol that would cyclize to the 5-membered ring derivative **7** even during handling. The MDM protecting group is labile enough to fall off under the chosen conditions,[11] but stable enough to make its handling more convenient.

Due to the relatively modest selectivity shown by the SSY library for **1**, we expected these peptides not to be efficient catalysts, but to perform the task to a certain degree. Binding constant determinations[10] revealed that the selected peptides are weak binders of the *N*-oxide, but still one order of magnitude better than **10**, a Lys-containing peptide not selected in our assays (Table 2).

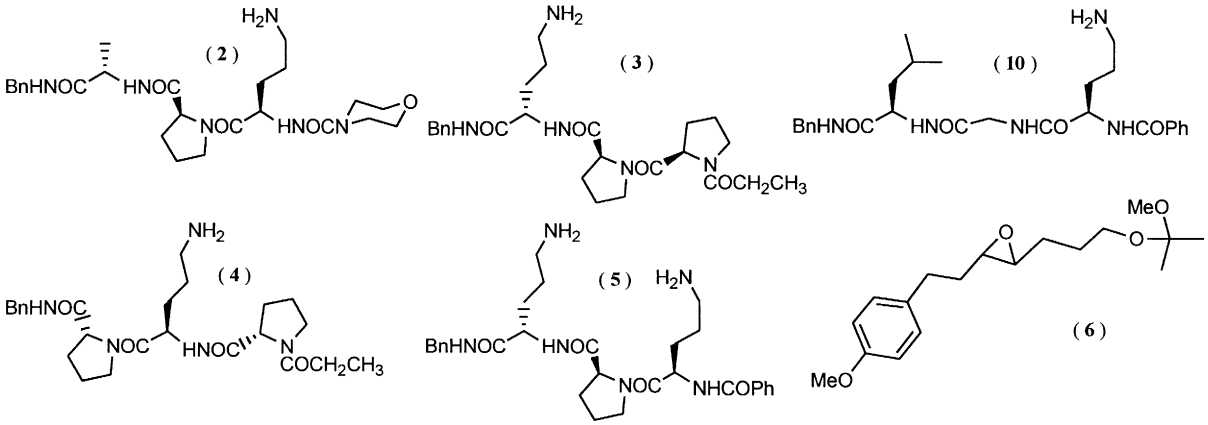


Fig. 4. Peptides re-synthesized in solution and the easier to handle epoxy-alcohol **6**

Table 2
Percentage of **8** formed when to a 2 mM solution of **6** where added: 2 mM peptide (1st and 2nd column); 10 mM peptide (3rd column); 20 mM peptide (4th column); A=$CHCl_3$:hexanes=2:1; B=$CHCl_3$:hexanes:THF=1:1:1

	A	B			K_a [M^{-1}]
(**2**)	11.6	12.7±0.4	10.8	11.2	56.1±0.2
(**3**)	13.1	14.0±0.6	13.6	13.6	35.6±0.1
(**4**)	13.2	14.3±0.5	12.2	12.1	41.5±0.0
(**5**)	1.9	3.6±0.5	NA	NA	NA
(**10**)	NA	0.9	NA	NA	3.7±0.5

Addition of the chosen peptides[11] to the solution of **6** increased the amount of the 6-membered derivative formed. Peptides **3** and **4** performed slightly better than peptide **2**. Addition of **5** to the protected epoxy-alcohol **6** produced no significant effect.[12] Additionally, the amount of **8** is somehow higher when THF is added to the assay solution (Table 2). In the control assay that contained **10**, a peptide not selected in our assays, about 1% of pyran derivative **8** was observed. These data correspond to a roughly 10–15 times increase in the amount of disfavored product resulted by the addition of the catalytic peptides. Although the study did not yet result in an efficient catalyst, it led however, to compounds with similar structural and functional characteristics that perform the requested task to a certain degree. These characteristics can now be assimilated into the construction of a directed structurally more rigid combinatorial library in the hope of yielding a much better catalyst.

Acknowledgements

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6. The initial concentration of *N*-oxide derivative **1** was 1 mM. The concentration was lowered each day by addition of solvent until the solution became colorless but some beads retained a red coloration. At the end of the assay the solution was removed and 20 μL of DMF were added to facilitate picking. Immediately after, the red beads were removed using capillary suction. Each was introduced in a 25 μL capillary in 1.5 μL DMF and photolyzed for 6 h under a UV lamp. Decoding was achieved as described in: Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. USA*. **1993**, 90, 10922.
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8. The acid fluoride method was used for amino acid coupling. After TFA deprotection, the peptides were run through a C18 column and reverted to the free amine by eluting on Sephadex-LH20 with methanol (1% TEA).
9. To 100 mg (0.45 mmols) alcohol were added 5 mL 2-methoxypropene, followed by a catalytic amount of TsOH·py. The solution was stirred at rt for 30 min and then filtered on a thin bed of basic alumina. Solvent removal gave 130 mg oil (99% yield). An amount of 50 mg (0.17 mmols) of this olefin was dissolved in 10 mL DCM and cooled to 0°C. Excess DMDO solution in acetone was added, and stirring continued for 30 min at 0°C and 30 min at rt. The solvent was removed to give the epoxide quantitatively as a colorless oil. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.18 (d, J=8.7 Hz, 2H), 6.88 (d, J=8.7 Hz, 2H), 3.78 (s, 3H), 3.42–3.36 (m, 2H), 3.14 (s, 3H), 2.72–2.62 (m, 4H), 1.84–1.74 (m, 2H), 1.66–1.52 (m, 4H), 1.25 (s, 6H). ^{13}C NMR (300 MHz, acetone-d_6): δ 158.5, 133.9, 129.6, 114.1, 60.1, 58.4, 57.8, 54.9, 47.8, 34.5, 31.5, 26.8, 24.2. LRMS (FAB): M=308 calculated for $C_{18}H_{28}O_4$. Found *m/z*=309 (M+1).
10. Binding constants were measured using NMR techniques. The differences in chemical shift observed at the addition of various amounts of *N*-oxide to a 0.02 M solution of peptide in $CDCl_3$, were quantified as described in: Connors, K. A. *Binding Constants: the Measurement of Molecular Complex Stability*; Wiley: New York, 1987; pp. 189–200. K_a for peptide **5** was not determined due to poor solubility in $CDCl_3$.
11. To solutions of 2 mM of **6** were added 2, 10 and 20 mM solutions of peptides, respectively, in a mixture of solvents. Each vial had added 5% v/v PIPES buffer 0.5 M pH=6.6. Measurements were performed only after 3 days of vigorous stirring at 70°C to assure the complete conversion of the protected epoxy-alcohol to the 5- and 6-membered ring products. After this period of time, no **9** or **6** were observed, the reaction media containing solely **7** and **8**. Aliquots were injected in HPLC. Separation of products was achieved on a silica gel column using a mixture of chloroform: methanol:hexanes:ethyl acetate=40:1:35:24, and the products were monitored at 277 nm. 4-Methoxybenzyl alcohol was employed as external standard.
12. Methanol (5% v/v) was added to the reaction mix to bring the peptide in solution.