ORGANIC LETTERS

2010
Vol. 12, No. 4
696−699

Convergent Synthesis of α-Ketoamide Inhibitors of Pin1

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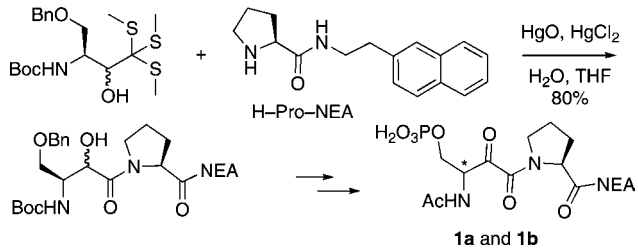
Received November 23, 2009

ABSTRACT



1a and **1b**

A convergent synthesis of α-ketoamide inhibitors of Pin1 is described. An α-hydroxyorthothioester derivative of Ser was reacted directly with an amine synthon. The reaction was catalyzed by HgO and $HgCl_2$ to form α-hydroxyamide. Thus, hydrolysis and coupling were combined in one step with 80% yield. Two diastereomers of a phospho-Ser-Pro α-ketoamide analogue were synthesized. The IC_{50} values of 100 and 200 μM were surprisingly weak for Pin1 peptidyl prolyl isomerase.

α-Ketoamides have been widely used in developing inhibitors of peptidases,[1] HDACs,[2,3] and peptidyl prolyl isomerases (PPIases).[4] One of the best studied immunosuppressant drugs, FK506, has an α-ketoamide, and it is a transition state analogue inhibitor of FK506 binding proteins (FKBPs).[5] α-Ketoamides have been used to inhibit several classes of proteases, such as serine proteases,[6−10] cysteine proteases,[11−14] and HIV and FIV proteases.[15]

α-Ketoamides have an electron-deficient carbonyl due to the effect of the neighboring electron-withdrawing amide. α-Ketoamide-containing inhibitors have been used to elucidate the mechanisms of enzymes because they serve as transition state analogues to inhibit enzyme catalytic activity. This occurs for serine or cysteine proteases through the formation of a tetrahedral intermediate analogue (*gem*-diol or hemiketal) with the enzyme upon binding.[16] FK506, a macrolide with an α-ketoamide functional group, has a dihedral angle of 95° to 100° between the two carbonyl

(1) Ocain, T. D.; Rich, D. H. *J. Med. Chem.* **1992**, *35*, 451–456.
(2) Curtin, M.; Glaser, K. *Curr. Med. Chem.* **2003**, *10*, 2373–2392.
(3) Wada, C. K.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Garland, R. B.; Holms, J. H.; Li, J.; Pease, L. J.; Guo, J.; Glaser, K. B.; Marcotte, P. A.; Richardson, P. L.; Murphy, S. S.; Bouska, J. J.; Tapang, P.; Magoc, T. J.; Albert, D. H.; Davidsen, S. K.; Michaelides, M. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3331–3335.
(4) Wang, X. J.; Etzkorn, F. A. *Biopolymers* **2006**, *84*, 125–146.
(5) Rosen, M. K.; Standaert, R. F.; Galat, A.; Nakatsuka, M.; Schreiber, S. L. *Science* **1990**, *248*, 863–866.
(6) Wipf, P.; Kim, H. *J. Org. Chem.* **1993**, *58*, 5592–5594.
(7) Maryanoff, B. E.; Zhang, H. C.; Greco, M. N.; Glover, K. A.; Kauffman, J. A.; Andrade-Gordon, P. *Bioorg. Med. Chem.* **1995**, *3*, 1025–1038.
(8) Ogilvie, W.; Bailey, M.; Poupart, M. A.; Abraham, A.; Bhavsar, A.; Bonneau, P.; Bordeleau, J.; Bousquet, Y.; Chabot, C.; Duceppe, J. S.; Fazal, G.; Goulet, S.; Grand-Maitre, C.; Guse, I.; Halmos, T.; Lavallée, P.; Leach, M.; Malenfant, E.; O'Meara, J.; Plante, R.; Plouffe, C.; Poirier, M.; Soucy, F.; Yoakim, C.; Déziel, R. *J. Med. Chem.* **1997**, *40*, 4113–4135.
(9) Wasserman, H. H.; Petersen, A. K. *J. Org. Chem.* **1997**, 8972–8973.
(10) Cacciola, J.; Fevig, J. M.; Stouten, P. F.; Alexander, R. S.; Knabb, R. M.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1253–1256.
(11) Li, Z.; Ortega-Vilain, A. C.; Patil, G. S.; Chu, D. L.; Foreman, J. E.; Eveleth, D. D.; Powers, J. C. *J. Med. Chem.* **1996**, *39*, 4089–4098.
(12) Choe, Y.; Brinen, L. S.; Price, M. S.; Engel, J. C.; Lange, M.; Grisostomi, C.; Weston, S. G.; Pallai, P. V.; Cheng, H.; Hardy, L. W.; Hartsough, D. S.; McMakin, M.; Tilton, R. F.; Baldino, C. M.; Craik, C. S. *Bioorg. Med. Chem.* **2005**, *13*, 2141–2156.
(13) Shirasaki, Y.; Miyashita, H.; Yamaguchi, M. *Bioorg. Med. Chem.* **2006**, *14*, 5691–5698.
(14) Barrett, D. G.; Catalano, J. G.; Deaton, D. N.; Hassell, A. M.; Long, S. T.; Miller, A. B.; Miller, L. R.; Ray, J. A.; Samano, V.; Shewchuk, L. M.; Wells-Knecht, K. J.; Willard, D. H., Jr.; Wright, L. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1735–1739.
(15) Slee, D. H.; Laslo, K. L.; Elder, J. H.; Ollmann, I. R.; Gustchina, A.; Kervinen, J.; Zdanov, A.; Wlodawer, A.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 11867–11878.

10.1021/ol9027013
Published on Web 01/26/2010

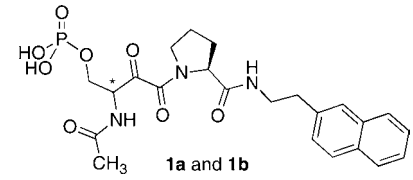
groups.[5] NMR studies of a ^{13}C-labeled structure and a crystal structure of FK506 showed that the two carbonyls were orthogonal both in bound and unbound structures, indicating that FK506 acts as a "twisted amide" transition state analogue to inhibit FKBPs.[5]

Pin1, a PPIase enzyme belonging to the parvulin family, specifically recognizes pSer/pThr-Pro containing substrates and regulates many cellular events such as cell cycle progression, transformation, and cell proliferation.[17,18] Pin1 is overexpressed in many cancer cell lines and plays an important role in oncogenesis.[19,20] Pin1 is a potential diagnostic and therapeutic anticancer target.[21−23] Thus, we are interested in developing potent and specific inhibitors of Pin1.[24,25]

Since α-ketoamides are an important class of enzyme inhibitors, numerous synthetic methods have been reported, including: cyano ylide coupling,[26] transition-metal catalysis,[27] amidation of α-keto acids with amines,[11,28] oxidation of α-hydroxyamides with Dess−Martin periodinane[6] or TEMPO,[29,30] and nucleophilic addition of a Grignard reagent to a Weinreb amide.[31] A solid-phase method for synthesis of α-ketoamides has been developed.[32] In this study, we have developed a convergent route to synthesize α-ketoamides by coupling a complex amine directly to an α-hydroxy-orthothioester to form an α-hydroxyamide, which was then oxidized to an α-ketoamide with the Dess−Martin periodinane.[33]

We have reviewed Pin1 inhibitors, including synthetic inhibitors, and natural products.[4,23] α-Ketoamides are an important class of inhibitor for PPIases (e.g., FK506). The cocrystal structure of FK506 with FKBP was the main evidence cited to support the "twisted amide" transition state for PPIases,[5,34] but α-ketoamides have not yet been reported as Pin1 inhibitors. Our impetus for synthesizing these challenging phosphorylated α-ketoamides was to investigate the ability of these compounds to inhibit Pin1, the only PPIase that regulates the cell cycle.

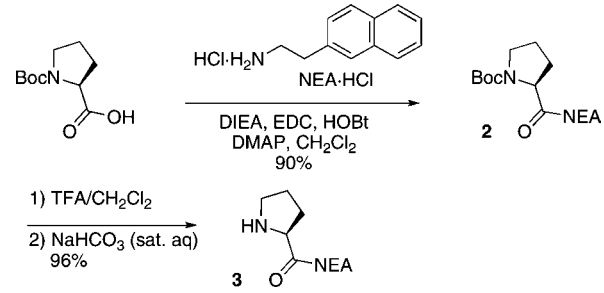
α-Ketoamides **1a** and **1b** were designed based on the specificity of Pin1 for the pSer/pThr-Pro motif.[18] An acetyl was chosen for the N-terminus, since cocrystal structures of Pin1-inhibitor complexes showed no electron density for N-terminal residues, and to improve water solubility over Fmoc derivatives.[25,35,36] An aromatic naphthyl was introduced at the C-terminus based on substrate and inhibitor sequence specificities.[18,36,37]



1a and **1b**

Boc-Pro-OH and 2-(2-naphthyl)-ethylamine (NEA) were coupled with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide•HCl (EDC) to give Boc-Pro-NEA **2** (Scheme 1). Free amine **3** was prepared by TFA deprotection of **2**, followed by neutralization with aqueous $NaHCO_3$.

Scheme 1. Synthesis of H-Pro-NEA **3**



Aldehyde **4** was obtained by $LiAlH_4$ reduction of the Weinreb amide of protected Ser (Scheme 2).[38] Lithio trismethylthiomethane was added to aldehyde **4** to give orthothioester **5**.[39] The two diastereomers of orthothioester **5** were not separated at this stage since the newly formed stereocenter would be eliminated in the subsequent oxidation of alcohol **7** to ketone **8**. Orthothioester **5** was converted directly to α-hydroxyamide **6** by aminolysis.

(16) Maryanoff, B. E.; Qiu, X.; Padmanabhan, K. P.; Tulinsky, A.; Almond, H. R., Jr.; Andrade-Gordon, P.; Greco, M. N.; Kauffman, J. A.; Nicolaou, K. C.; Liu, A.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8048–8052.

(17) Lu, K. P.; Hanes, S. D.; Hunter, T. *Nature* **1996**, *380*, 544–547.

(18) Yaffe, M. B.; Schutkowski, M.; Shen, M.; Zhou, X. Z.; Stukenberg, P. T.; Rahfeld, J. U.; Xu, J.; Kuang, J.; Kirschner, M. W.; Fischer, G.; Cantley, L. C.; Lu, K. P. *Science* **1997**, *278*, 1957–1960.

(19) Bao, L.; Kimzey, A.; Sauter, G.; Sowadski, J. M.; Lu, K. P.; Wang, D. G. *Am. J. Pathol.* **2004**, *164*, 1727–1737.

(20) Ryo, A.; Liou, Y. C.; Lu, K. P.; Wulf, G. *J. Cell Sci.* **2003**, *116*, 773–783.

(21) Finn, G.; Lu, K. P. *Curr. Cancer Drug Targets* **2008**, *8*, 223–229.

(22) Lu, K. P. *Cancer Cell* **2003**, *4*, 175–180.

(23) Xu, G. G.; Etzkorn, F. A. *Drug News Perspect.* **2009**, *22*, 399–407.

(24) Wang, X. J.; Xu, B.; Mullins, A. B.; Neiler, F. K.; Etzkorn, F. A. *J. Am. Chem. Soc.* **2004**, *126*, 15533–15542.

(25) Zhao, S.; Etzkorn, F. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6615–6618.

(26) Wasserman, H. H.; Ho, W.-B. *J. Org. Chem.* **1994**, *59*, 4364–4366.

(27) Ozawa, F.; Soyama, H.; Yanagihara, H.; Aoyama, I.; Takino, H.; Izawa, K.; Yamamoto, T.; Yamamoto, A. *J. Am. Chem. Soc.* **1985**, *107*, 3235–3245.

(28) Chiou, A.; Verger, R.; Kokotos, G. *Lipids* **2001**, *36*, 535–542.

(29) Harbeson, S. L.; Abelleira, S. M.; Akiyama, A.; Barrett, R.; Carroll, R. M.; Straub, J. A.; Tkacz, J. N.; Wu, C.; Musso, G. F. *J. Med. Chem.* **1994**, *37*, 2918–2929.

(30) Han, W.; Jiang, X.; Hu, Z.; Wasserman, Z. R.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3487–3490.

(31) Sibi, M. P.; Marvin, M.; Sharma, R. *J. Org. Chem.* **1995**, *60*, 5016–5023.

(32) Papanikos, A.; Meldal, M. *J. Comb. Chem.* **2004**, *6*, 181–195.

(33) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.

(34) Fischer, S.; Michnick, S.; Karplus, M. *Biochemistry* **1993**, *32*, 13830–13837.

(35) Etzkorn, F. A.; Noel, J. P.; Zhang, Y.; Wang, X. J. *Understanding Biology Using Peptides, Proc. 19th Am. Pept. Symp.*; Blondelle, S. E., Ed.; Springer, Inc.: San Diego, 2006; pp 759−762.

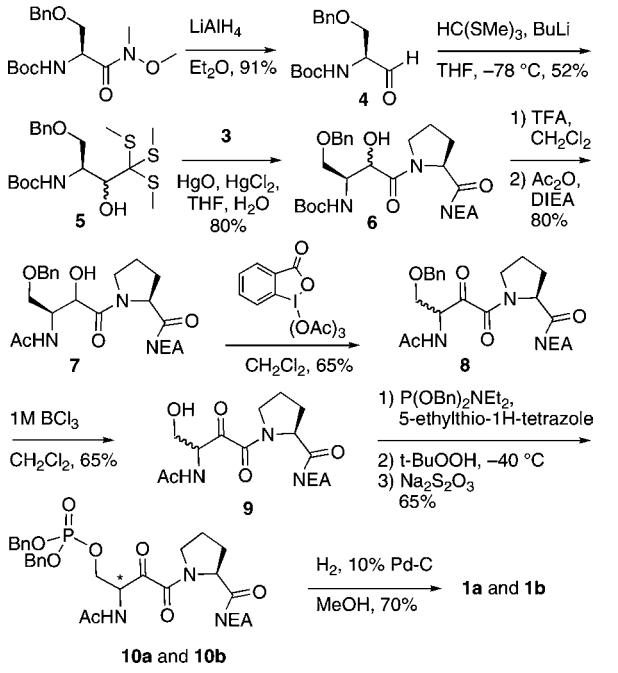
(36) Zhang, Y.; Daum, S.; Wildemann, D.; Zhou, X. Z.; Verdecia, M. A.; Bowman, M. E.; Lucke, C.; Hunter, T.; Lu, K. P.; Fischer, G.; Noel, J. P. *ACS Chem. Biol.* **2007**, *2*, 320–328.

(37) Wildemann, D.; Erdmann, F.; Alvarez, B. H.; Stoller, G.; Zhou, X. Z.; Fanghanel, J.; Schutkowski, M.; Lu, K. P.; Fischer, G. *J. Med. Chem.* **2006**, *49*, 2147–2150.

(38) Wang, X. J.; Hart, S. A.; Xu, B.; Mason, M. D.; Goodell, J. R.; Etzkorn, F. A. *J. Org. Chem.* **2003**, *68*, 2343–2349.

(39) Hu, Z.; Han, W. *Tetrahedron Lett.* **2008**, 901–902.

Scheme 2. Synthesis of Inhibitors **1a** and **1b**



The Boc group of **6** was removed with TFA, and the resulting amine was selectively acetylated with 1 equiv of acetic anhydride in the presence of the free hydroxyl to give **7** (Scheme 2). Alcohol **7** was oxidized with the Dess−Martin reagent to form α-ketoamide **8**.[6,33] Although the Dess−Martin oxidation is known to produce the least epimerization alpha to electrophilic ketones,[6,40,41] epimerization occurred either during oxidation or chromatography, but the diastereomers (1:1) were not separated at this stage. This epimerization occurs easily due to the strongly electron-withdrawing ketoamide group and has been observed in several syntheses of α-ketoamides.[15,29] Epimerization of α-ketoesters has been observed to occur in solution upon standing for one week.[41] Both phospho-L-Thr and phospho-D-Thr containing inhibitors of Pin1 have been reported, and the D-Thr inhibitors were more potent.[36,37] We wanted to test both diastereomers of **1** for inhibition, so we did not attempt to improve the stereochemical purity of α-ketoamide **8**.

The benzyl on the side chain of serine was removed with BCl_3 to give alcohol **9**.[42,43] Reverse-phase, semipreparative HPLC purification gave a mixture of diastereomers of **9** in a 1:1 ratio as determined by ^{1}H NMR (Supporting Information). The 86 ppm ^{13}C NMR peak was assigned as the hydrated ketone carbon based on the literature and the assignment below for **10a**.[1] The ketone carbonyl stretch did not appear in the IR at ca. 1720 cm^{-1}, which also indicates hydration of the ketone.

Alcohol **9** was phosphorylated with dibenzyl *N*,*N*-diethylphosphoramidite, followed by oxidation with *t*-BuOOH to produce dibenzylphosphate **10**.[24,44] The diastereomers of **10** were isolated by reverse-phase semipreparative HPLC. The ketone carbonyl for both diastereomers **10a** and **10b** appeared in the IR, but not in the ^{13}C NMR. The 84 ppm peak in the ^{13}C and the 4.74 ppm peak in the ^{1}H NMR were assigned by HMBC as the hydrated ketone (Supporting Information). The ketone carbonyl stretch appeared in the IR at 1723 cm^{-1}, but much weaker than expected, indicating partial hydration.

Hydrogenolysis of each diastereomer of **10** under neutral conditions furnished two diastereomers of the final compound, **1a** and **1b**. Compounds **1a** and **1b** showed no ketone carbonyl in both the ^{13}C and IR spectra. Since we found that both diastereomers were weak Pin1 inhibitors, vide infra, we chose not to determine the absolute stereochemistry of the inhibitors.

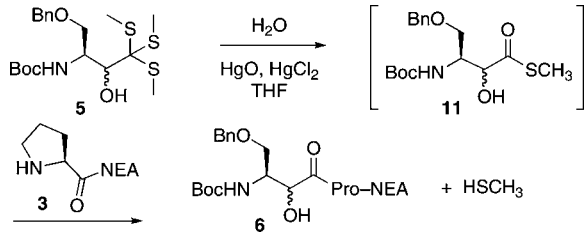
In our method, orthothioester **5** was directly reacted with the fully formed right half of the molecule, the amine H-Pro-NEA **3**, as the nucleophile to produce amide **6** (Scheme 2). Orthothioester **5** was used as a carboxylic acid synthon instead of a nitrile to avoid the toxicity of NaCN or KCN. In addition, conditions for the hydrolysis of a nitrile are incompatible with amides.[29]

This synthesis of the target compound was convergent compared with previous linear syntheses of α-ketoamides via α-hydroxy acids. Previously, orthothioesters were either hydrolyzed to a carboxylic acid and coupled with an amine or methanolyzed to a methyl ester.[39,45,46] Methyl esters require the additional step of hydrolysis before coupling, while α-hydroxy acids are difficult to isolate and purify. Instead, the H-Pro-NEA amine was coupled in one step to the α-hydroxyorthothioester. We attempted ozonolysis of a cyano ylide to form the α-ketoamide functionality using Wasserman's method.[9] However, due to the instability of the α-ketoamide in subsequent Pro ester hydrolysis and coupling to NEA, we chose to use aminolysis of thioester **5** with amine **3** to form the complete skeleton prior to oxidation to the ketoamide.

The aminolysis was catalyzed by HgO and $HgCl_2$, similar to hydrolysis of orthothioesters.[39,45] Thus, the α-hydroxyamide **6** was formed without additional coupling reagents or steps. We propose that the key step in the reaction involves Hg(II)-catalyzed hydrolysis of the orthothioester to a methyl thioester intermediate **11** (Scheme 3).[45,47] The THF used as the solvent was wet, which provided the water for the hydrolysis. Aminolysis with the amine nucleophile present in situ then gave amide **6** directly. Thus, hydrolysis and coupling were combined in a single step with 80% yield. Although the overall yield in this 12-step synthesis is not high (6% for the 9 linear steps), the aminolysis to form the

(40) Burkhart, J. P.; Peet, N. P.; Bey, P. *Tetrahedron Lett.* **1988**, *29*, 3433–3436.
(41) Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E.; Free, C. A.; Smith, S. A.; Petrillo, E. W., Jr. *J. Med. Chem.* **1993**, *36*, 2431–2447.
(42) Williams, D. R.; Brown, D. L.; Benbow, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 1923–1925.
(43) Xie, J.; Menand, M.; Valery, J.-M. *Carbohydr. Res.* **2005**, *340*, 481–487.
(44) de Bont, H. B.; Liskamp, R. M.; O'Brian, C. A.; Erkelens, C.; Veeneman, G. H.; van Boom, J. H. *Int. J. Pept. Protein Res.* **1989**, *33*, 115–123.
(45) Mamane, V.; Aubert, E.; Fort, Y. *J. Org. Chem.* **2007**, *72*, 7294–7300.
(46) Braukmüller, S.; Brückner, R. *Eur. J. Org. Chem.* **2006**, 2110–2118.
(47) Orito, K.; Seki, Y.; Suginome, H.; Iwadare, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 2013–2017.

Scheme 3. Proposed Mechanism for Hydroxyamide **6** Formation



key intermediate for α-ketoamides should be very useful in a variety of syntheses.

The α-chymotrypsin protease-coupled assay, with succinyl-Ala-Glu-*cis*-Pro-Phe-*p*-nitroanilide (*p*NA) as the substrate, was used to measure inhibition of Pin1 by the two diastereomers of **1**.[24] The absorbance of *p*NA released from the PPIase trans product by chymotrypsin was recorded continuously at 390 nm by UV/vis. The IC_{50} values of the two diastereomers were determined to be 100 ± 20 μM and 200 ± 20 μM.

Surprisingly, neither diastereomer was a potent inhibitor of Pin1. These inhibitors were less potent than a similarly substituted, ground-state analogue, Fmoc-pSer-Ψ[(*Z*)CH═C]-Pro-(2)-*N*-(3)-ethylaminoindole ($IC_{50} = 28$ μM).[25] Since the α-ketoamide of FK506 acts as a transition state analogue to inhibit FKBPs,[5] we expected more potent inhibition of Pin1 by at least one of the α-ketoamide stereoisomers. There are two proposed mechanisms for Pin1 catalysis: the twisted-amide mechanism[5,48,49] and the Cys-113 tetrahedral intermediate mechanism.[50] The hemiketal of an α-ketoamide has been shown to act as a tetrahedral transition-state analogue for a serine protease.[16] We anticipated that the ketoamide could act as a potent electrophile if the Cys-113 thiol of Pin1 was positioned for nucleophilic addition. The twisted amide mechanism is not supported by this poor inhibition either. Although a hydrated ketone would preclude either Cys-113 nucleophilic addition or the orthogonal carbonyl conformation necessary to mimic the twisted amide transition state, as in FK506, the hydration is reversible. The ketone state should be accessible for binding to the enzyme if it were a favorable analogue of the transition state.

Although the two diastereomers of ketoamides were weak inhibitors of Pin1, we have provided a convergent method for synthesizing α-ketoamide peptidomimetics, which is a very useful class of enzyme inhibitors, especially for proteases. Many α-ketoamides have been developed as drugs, and our convergent method contributes to the available methods for their synthesis.

Acknowledgment. We thank the NIH for Grant No. R01 CA110940 for financial support.

Supporting Information Available: Experimental procedures, HPLC, and spectroscopic data for compounds **1**−**10** and inhibition data for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL9027013

(48) Schroeder, O. E.; Carper, E.; Wind, J. J.; Poutsma, J. L.; Etzkorn, F. A.; Poutsma, J. C. *J. Phys. Chem. A* **2006**, *110*, 6522–6530.

(49) Wiederrecht, G.; Etzkorn, F. A. *Perspect. Drug Discovery Des.* **1994**, *2*, 57–84.

(50) Ranganathan, R.; Lu, K. P.; Hunter, T.; Noel, J. P. *Cell* **1997**, *89*, 875–886.