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Evidence for Separate Binding and Scaffolding Domains in the Immunosuppressive and Antitumor Marine Natural Product, Pateamine A: Design, Synthesis, and Activity Studies Leading to a Potent Simplified Derivative

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Abstract: Pateamine A (PatA), a marine metabolite from *Mycale* sp., is a potent inhibitor of the intracellular signal transduction pathway emanating from the T-cell receptor leading to the transcription of cytokines such as interleukin-2 (IL-2). On the basis of the structure of PatA and initial biological results, a hypothesis was developed regarding the presence of distinct binding and scaffolding domains in the PatA structure with respect to interactions with its putative cellular receptor(s). Employing a highly convergent approach involving a Hantzsch coupling strategy, we probed this hypothesis by preparing a simplified PatA derivative (desmethyl, desamino PatA, DMDAPatA, **3**). This derivative was prepared in 10 fewer synthetic steps relative to PatA and was indeed found to exhibit equal to greater potency (IC_{50} 0.81 ± 0.27 nM) in inhibition of IL-2 production relative to PatA (IC_{50} 4.01 ± 0.94 nM) thus providing support for the binding/scaffolding domain hypothesis. In addition, as a means to find more stable derivatives and gain further insights into structure−activity relationships, several PatA derivatives were synthesized and assayed in the IL-2 reporter gene assay. Several of these derivatives displayed lower potency but marked stability relative to the natural product and provide further insights into the nature of the binding domain required for activity.

Introduction

Natural products have proven to be extremely useful as probes of biological processes.[1] Examples include the immunosuppressive, microbial secondary metabolites, cyclosporin A, FK506, and rapamycin.[2] Marine organisms are a rich source of bioactive compounds, and many of these complex metabolites are proving useful as drug leads and biological probes.[3] For example, bryostatin,[4] epothilone,[5] discodermolide,[6] and ecteinascidin[7] show great potential as anticancer agents and have revealed interesting biological mechanisms of action. An important recent discovery is the marine agent pateamine A (PatA (**1**), Figure 1), isolated from the *Mycale* sp.,[8] which is showing great promise as a biological probe of the complex intracellular signaling pathways involved in T-cell activation.[9] Preliminary studies by a group at PharmaMar showed potent activity of PatA in the mixed lymphocyte reaction and also in the mouse skin graft rejection assay.[10] More recent studies from our laboratories suggest that PatA inhibits a specific intracellular signaling pathway involved in T-cell receptor-mediated IL-2 production.[11] In addition to its effect on TCR signaling pathway, PatA was also found to induce apoptosis in certain mammalian cell lines, especially those that are transformed with the oncogene Ras.[12]

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§ Work performed while on sabbatical from Chong Kun Dang Research Institute (S. Korea).
|| Current address: Pfizer, St. Louis, MO.

(1) Schreiber, S. L.; Hung, D. T.; Jamison, T. F. *Chem. Biol.* **1996,** *3*, 623−639.
(2) (a) For a review on immunosuppressive natural products, see: (b) Hung, D. T.; Jamison, T. F.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 623−639.
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(8) Northcote, P. T.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron Lett.* **1991**, *32*, 6411. This initial communication of pateamine A isolation and 2D structure elucidation described selective cytotoxic and antifungal activity.
(9) Alexander Akhiezer, Ph.D. Thesis, Massachusetts Institute of Technology, 1999.
(10) Immunosuppressive activity was first discovered by Dr. Glynn Faircloth, PharmaMar Inc., Cambridge, MA (private communication). PatA showed potent activity in the mixed lymphocyte reaction, (IC_{50} 2.6 nM) and in the mouse skin graft rejection assay, PatA was found to be more potent than cyclosporin A with only low toxicity at high doses but all doses were active.
(11) Romo, D.; Rzasa, R. M.; Shea, H. A.; Park, K.; Langenhan, J. M.; Sun, L.; Akhiezer, A.; Liu, J. O. *J. Am. Chem. Soc.* **1998**, *120*, 12 237−12 254.
(12) Hood, K. A.; West, L. M.; Northcote, P. T.; Berridge, M. V.; Miller, J. H. *Apoptosis* **2001**, *6*, 207−219.

10.1021/ja040065s CCC: $27.50

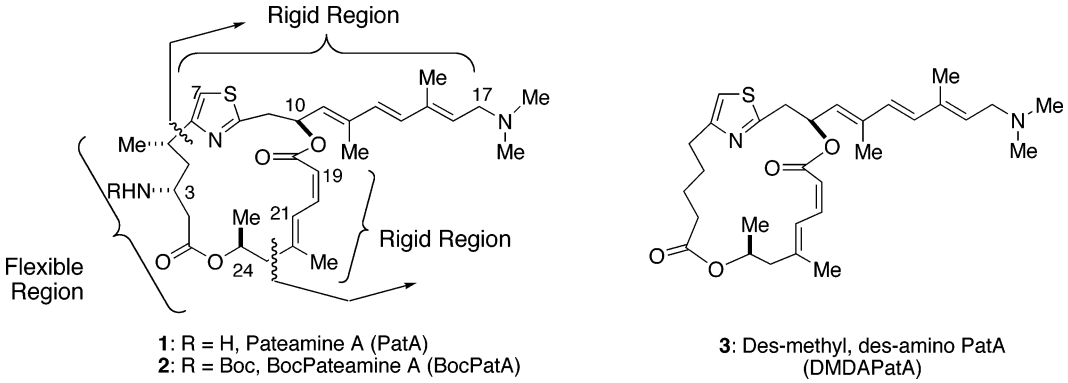


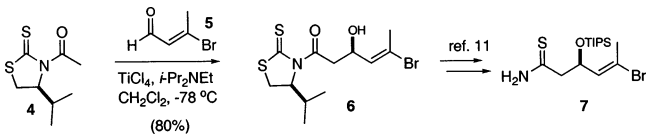
Figure 1. Structures of pateamine A (**1**), Boc-pateamine A (**2**), and des-methyl, des-amino pateamine A (DMDAPatA, **3**). Also shown are the putative binding (rigid regions) and scaffolding (flexible) domains.

Analysis of the PatA structure reveals a rigid eastern half (C6−C24) including the thiazole, dienoate, and the triene side chain, due to extended conjugation, and a more flexible western half (C1−C5). Furthermore, C3−Boc-PatA was found to have only 3−4-fold lower activity than PatA.[11] These facts in conjunction with simple molecular modeling studies led us to hypothesize that PatA consists of separate binding (C6−C24) and scaffolding (C1−C5) domains (Figure 1). Herein, we describe support for this hypothesis garnered from the total synthesis and biological analysis of a simplified derivative, devoid of the C3-amino and C5-methyl groups (**3**, DMDAPatA) that builds on our previously described total synthesis but importantly includes improvements to several reaction steps leading to a more practical synthesis.[11] For example, the synthesis of DMDAPatA incorporates a practical, convergency-building strategy involving a Hantzsch thiazole coupling. We also describe the synthesis and biological testing of PatA derivatives that were designed to impart stability to the labile PatA structure. In particular, the goal was to stabilize the acid sensitive triallylic acetate moiety (i.e., C10 position).

Results and Discussion

Structural Analysis of PatA and DMDAPatA. Inspection of the PatA structure reveals a highly unsaturated C6−C24 region consisting of a thiazole, a hydroxy dienoate, and a triene. In addition, a more flexible region (C1−C5) bearing amino and methyl substituted stereogenic carbons is found in the macrocycle. This simple and cursory structural analysis and simple molecular modeling studies in combination with the previously reported potent biological activity of an acylated C3-amine derivative (e.g., **2**, R = Boc)[11] led us to a simple hypothesis. Does PatA possess separate binding (C6−C24) and scaffolding (C1−C5) domains? This notion was bolstered by consideration of the immunosuppressive agents CsA, FK506, and rapamycin, each of which has been functionally dissected into two structural domains responsible for interacting with distinct protein targets.[2] Many protein ligands are known to change conformations on binding to their receptors or alternatively, the binding event leads to a more defined conformation.[13] However, considering the preliminary biological data and simple structural analysis described above, we set out to synthesize the simplified PatA derivative **3** (DMDAPatA) devoid of the C3-amino and C5-methyl groups to provide evidence for this hypothesis and potentially arrive at a more readily prepared PatA derivative useful as a potential immunosuppressive or anticancer agent and also as a probe for studying the T-cell receptor mediated signaling pathway.

Scheme 1



Synthesis of PatA Derivatives. For the synthesis of DMDAPatA **3**, we returned to a strategy that we had considered and attempted for the total synthesis of PatA but were unable to bring to fruition.[14] This entailed a more convergent strategy to the C1−12 thiazole-containing fragment employing a Hantzsch coupling reaction (Scheme 2, **10** → **11** → **12**). Pattenden made use of a related-strategy in his synthesis of PatA.[15]

Since our initially reported total synthesis of PatA, we have found a more practical approach to the acetate aldol adduct **6** previously described, employing the modified Nagao method of Vilarrasa and co-workers.[16] Use of the same substrates, but employing $TiCl_4$ as Lewis acid, led to a more practical and scaleable procedure which could be readily performed on multigram scale to give the β-hydroxy amide **6** (Scheme 1). Subsequent conversion to the thioamide **7** was performed as previously described.[11]

The requisite α-bromoketone **10** required for Hantzch coupling was obtained by esterification and bromination of 6-oxoheptanoic acid (**8**). Hantzsch thiazole coupling between bromoketone **10** and thioamide **7**[11] using modified Meyers' conditions[17] provided thiazole **12** in good overall yield (62%). A critical prerequisite for optimal yields in this coupling was purification of the intermediate thiazoline **11** prior to the dehydration step in contrast to our previous applications of this reaction, in which this process could be performed in a single pot[11] and as previously described by Meyers.[17] Deprotection of the TIPS ether followed by a Mitsunobu coupling with the more robust TIPS protected version of the previously described enyne acid **14**[11] gave the macrocyclic precursor **15.** Deprotection

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(14) Unpublished results of Drs. Robert Rzasa and William D. Schmitz (TAMU).
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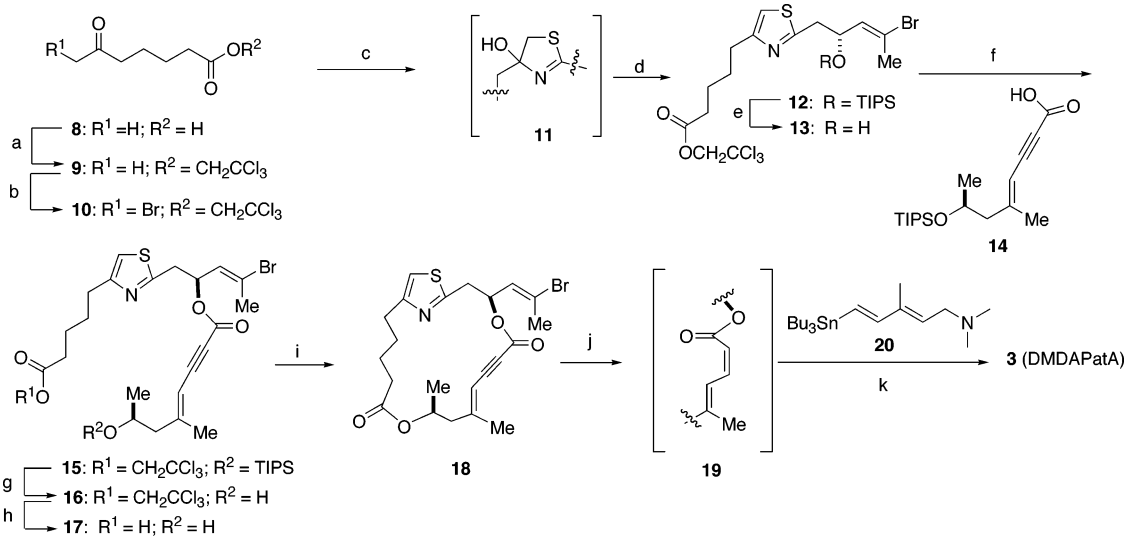
(16) González, A.; Aiguadé, J.; Urpí, F.; Vilarrasa, J. *Tetrahedron Lett.* **1996**, *37*, 8949−8952.
(17) Aguilar, E.; Meyers, A. I. *Tetrahedron Lett.* **1994**, *35*, 2473−2476.

Scheme 2



(a) $HOCH_2CCl_3$, $SOCl_2$, benzene, reflux, 5 h, 61%; (b) (i) TMSOTf, *i*-Pr_2NEt, 0 °C,1.5 h (ii) NBS, $NaHCO_3$, -78 °C, 2 h (79%, 2 steps); (c) 7, 2,6-lut., CH_2Cl_2, 25 °C, 18 h; (d) TFAA, py., Hünig's base, 0→25 °C, 3 h, 62% (2 steps); (e) TBAF, 20 mol % AcOH, THF, −20 °C, 3 h, 97%; (f) PPh_3, DIAD, THF, -20 °C, 1 h, 91%; (g) TBAF, 50 mol% AcOH, THF, 25 °C, 8 h, 80%; (h) 10% Cd/Pb, THF/1M NH_4OAc, 25 °C, 3 h, 89%; (i) 2,4,6-trichlorobenzoyl Chloride, Et_3N, DMAP, toluene, THF (0.001 M), 25 °C, 2 h, 84%; (j) $Pd(CaCO_3)$/Pb, H_2, MeOH, 25 °C, 3 h, 56%; (k) 10 mol % [Pd_2dba_3·$CHCl_3$: $AsPPh_3$=1:8], 20, THF, 25 °C, 14 h, 76%.

of the TIPS ether and trichloroethyl ester[18] of diester **15** followed by Yamaguchi macrocyclization[19] gave bis-macrolactone **18**. Lindlar reduction gave *E*,*Z*-diene **19**, and following a Stille coupling[20] with the previously described dienyl stannane **20**,[11] DMDAPatA (**3**) was obtained in 14 steps (longest linear sequence) from aldehyde **5**.

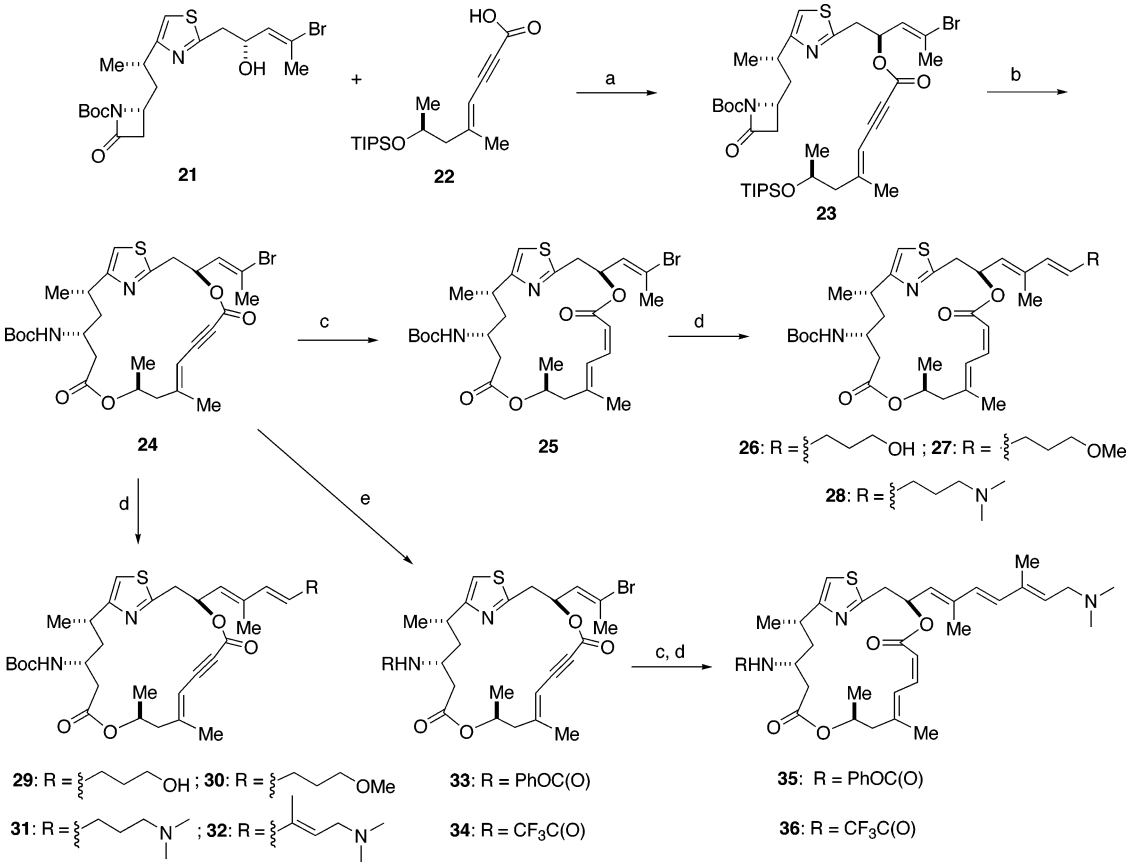
The synthesis of additional PatA derivatives with only minor structural variations began with the previously described β-lactam **21** (Scheme 3). The synthesis of all derivatives in this series followed a process similar to that previously employed in the total synthesis of PatA.[11] We found it best to introduce the side chain via a Stille coupling reaction as the final step in the synthesis due partly to the polarity introduced by the tertiary amine but primarily due to the instability associated with the triallylic ester moiety (i.e., C10, PatA numbering). Derivatives **26**−**31** were prepared to determine the structural tolerance of the terminal functional group on the side chain of PatA and also to potentially improve the stability of the acid labile triallylic acetate moiety by removal of one unsaturation. For this purpose, the required stannanes **37**−**39** for side chain-modified PatA derivatives were prepared by standard conditions and stannylcupration was performed as described previously for vinyl stannane **20** (Scheme 4).[11] The C3−Boc protecting group had previously been found to have a minor effect on activity (3−4-fold decrease in activity)[11] and improve stability and therefore, for ease of handling, this group was retained in all derivatives. PatA derivatives with a diene rather than a triene side chain bearing a hydrogen bond donor (i.e., dienyl alcohol **26)**, a less basic hydrogen bond acceptor (i.e., methyl ether **27)**, and the parent dimethyl amino group (i.e., dienyl dimethylamine **28)** were also synthesized. In addition, derivative **25** bearing the identical side-chain found in PatA with the exception of one unsaturation and the C16 methyl group was prepared. The effects on biological activity of a more rigid macrocycle (i.e., enyne vs dienoate) for BocPatA (i.e., enyne **32**) in conjunction with modified terminal functional groups on the diene were also investigated (i.e., enynes **29**-**31)**. These derivatives were readily prepared by omission of the Lindlar reduction step (Scheme 3).

Due to the aforementioned activity of C3−Boc PatA,[11] two additional C3-acyl amino derivatives were prepared with the expectation that they should have similar potency. In this regard, the C3-phenyl carbamate **35** and the C3-trifluoroacetamide **36** were synthesized by deprotection of Boc-macrocycle **24** followed by acylation to give macrocycles **33** and **34** (Scheme 3). Subsequent Lindlar reduction and Stille coupling gave the C3-acylated derivatives **35** and **36**.

The ability of these derivatives to inhibit T-cell receptor-mediated IL-2 production was analyzed using an IL-2 reporter gene assay. In this assay, a plasmid encoding a reporter gene (luciferase) under the control of the IL-2 promoter was first introduced into Jurkat T cells by transfection. The transfected Jurkat T cells are then stimulated with two pharmacological agents, phorbol myristyl acetate (PMA), which activates protein kinase C, and ionomycin, which allows calcium to enter T cells leading to activation of calmodulin and calcineurin. Together, PMA and ionomycin recapitulate T-cell receptor signaling, leading to the activation of the luciferase reporter gene by activating the IL-2 promoter. Concurrent with the IL-2 luciferease reporter gene, we also transfected the same population of Jurkat T cells with another plasmid encoding a constitutively expressed β-galactosidase as an internal control. The ability of PatA and its analogues to block T cell receptor-mediated IL-2 expression was determined after normalization of the IL-2 luciferase activity against the β-galactosidase activity from the same sample. As β-galactosidase gene expression is constitutive and not affected by stimulation through the T cell receptor, the normalization against β-galactosidase activity excludes the

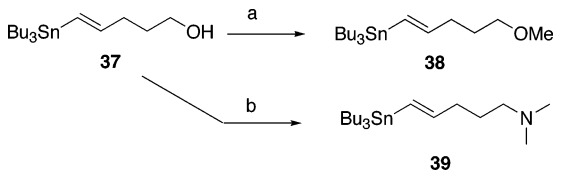
(18) Dong, Q.; Anderson, C. E.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 5681−5682.
(19) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Chem. Soc. Jpn.* **1979**, *52*, 1989−1993.
(20) Farina, V.; Krishnan, B. *J. Am. Chem. Soc.* **1991**, *113*, 9585−9595.

Scheme 3



(a) PPh_3, DIAD, THF, -20 °C, 2H, 71%; (b) (i) HF·py. THF, 25 °C, 24 h, 86%, (ii) Et_4NCN, CH_2Cl_2, 25 °C, 5 h, 65%; (c) $Pd(CaCO_3)$/Pb, H_2, MeOH, 12 h, 99% (d) 20 or 37−39, 10 mol % [Pd_2dba_3·$CHCl_3$: $AsPh_3$ = 1:8], THF, 25 °C, 10−18 h, 11−76%; (e) (i) 20% TFA, CH_2Cl_2, 0 °C, 15 h, 95%, (ii) PhCOCl or $(CF_3CO)_2O$, DMAP, py., CH_2Cl_2, 25 °C, 5 h, 99%.

Scheme 4



(a) Ag_2O, MeI, CH_3CN, reflux, 9 h, 25%; (b) (i) TsCl, py., CH_2Cl_2 0 °C, 8 h, 65% (ii) dimethyl amine (g), THF, −78 °C, 6 h, 85%.

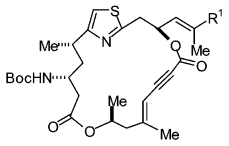
possibility that inhibition of IL-2 reporter gene is due to nonspecific cytotoxic effects to cells.[21]

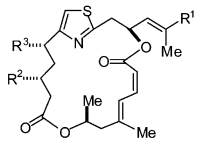
As seen in Table 1, most derivatives were in general less potent than PatA (**1**). As expected, the C3-phenyl carbamate derivative **35** possessed an activity that is approximately 4-fold lower than PatA (4 nM) in analogy to BocPatA (**3**). What is not readily explained is the reduced activity of the trifluoroacetamide **36** (∼303 nM). While we cannot offer an explanation at this time, we note that introduction of fluorine atoms into protein ligands often lead to results that are not readily rationalized.[22] A possible trend is observed upon comparison of dienoate macrocycles **26**−**28** and enynoate macrocycles **29**−**32**. Enyne derivatives having an expectedly more rigid macrocycle than the natural product and bearing oxygen rather than nitrogen at the terminus of the side chain (i.e., **29** and **30**) were found to have activities in the IL-2 reporter gene assay ranging between 55 and 335 nM, respectively. However, enyne derivatives **31** and **32** with side chains more closely resembling the natural product (i.e., amino end groups) had no activity. Conversely, the dienoate derivatives (i.e., **26** and **27**) that should have macrocyclic conformations similar to the natural product but bearing oxygen rather than nitrogen at the terminus of the side chain had very low activity. However, once nitrogen is introduced into the side chain, as in derivative **28**, activity is restored (328 nM) to some degree. Thus, it would appear that an oxygenated side-chain, regardless of hydrogen bonding capabilities, compensates to some extent for the change in macrocycle conformation that occurs upon introduction of an enyne. However, oxygen rather than nitrogen on the side chain leads to greatly diminished activity when coupled to the natural dienoate-containing macrocycle. Further analysis and understanding of these results in regard to their relevance for protein ligand interactions must await structural characterization of the interactions of the putative cellular protein receptor(s) with these PatA derivatives.

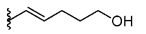
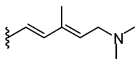
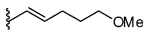
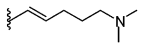
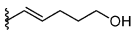
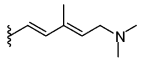
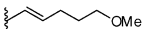
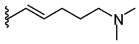
The most intriguing derivative and one that provides direct support for the binding/scaffolding hypothesis was DMDAPatA **3**. This derivative displayed similar to greater potency (IC_{50} 0.8 ± 0.3 nM) relative to PatA (IC_{50} 4.0 ± 0.9 nM) in its ability to inhibit expression of the IL-2 reporter gene in stimulated Jurkat T cells (Table 1). To ensure that the chemical modifications in DMDAPatA did not change its mechanism of action, we

(21) Su, B.; Jacinto, E.; Hibi, M.; Kallunki, T.; Karin, M.; Ben-Neriah, Y. *Cell* **1994**, *77*, 727−736.
(22) Filler, R., Kobayashi, Y., Yagupolskii, L. M., Eds. *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications*; Elsevier: Amsterdam, 1993.

Table 1. IL-2 Reporter Gene Assay (transfected Jurkat cells) Activity of Pateamine A and Derivatives





cmpd.	R^1	IC_{50} (nM)	cmpd.	R^1	R^2	R^3	IC_{50} (nM)
29		340±180	Pat A (**1**)		NH_2	Me	4.0±0.94
30		55±16	**3**	"	H	H	0.81±0.27
31		NA[a]	**26**		NHBoc	Me	>1000[b]
32		NA[a]	**27**		NHBoc	Me	>1000[b]
			28		NHBoc	Me	330±120
			35	"	NHC(O)OPh	Me	15±6.1
			36	"	$NHC(O)CF_3$	Me	300±93

[a] Not active. [b] Inhibition activity was observed, but it did not reach 50% even with the highest concentration tested. [c] It should be noted that the IC_{50} value for PatA in this particular assay is 10 fold higher than that previously reported (ref 11). It seems that Jurkat cells appear to vary in their sensitivity to PatA, depending in part on the number of passages they have undergone. All IC_{50} values listed in this table were determined using the same population of Jurkat T cells.

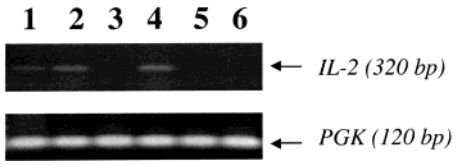


Figure 2. Reverse transcription-polymerase chain reaction assay to determine the level of mRNA for both IL-2 and PGK genes in the presence and absence of PatA and DMDAPatA. Lane 1, PMA + ionomycin; 2, PMA + ionomycin + 0.1 nM PatA; 3, PMA + ionomycin + 5 nM PatA; 4, PMA + ionomycin + 0.1 nM DMDAPatA; 5, PMA + ionomycin + 5 nM DMDAPatA; 6. DMSO (carrier solvent control).

determined the effects of PatA and DMDAPatA in an orthogonal assay, namely transcription of the endogenous IL-2 gene and also the housekeeping gene PGK in response to stimulation by PMA and ionomycin using a reverse-transcription-polymerase chain reaction (RT-PCR) assay. As shown in Figure 2, IL-2 mRNA is induced by treatment of Jurkat T cells with PMA plus ionomycin, similar to the induction of the IL-2 luciferase reporter gene (Lane 1). Treatment with sub-optimal doses of either PatA or DMDAPatA had no effect on the transcription of the IL-2 gene (Figure 2, Lanes 2 and 4). In the presence of higher concentrations of both PatA and DMDAPatA, transcription of endogenous IL-2 gene is completely inhibited (Figure 2, Lanes 3 and 5), with the level of IL-2 mRNA similar to cells without stimulation by PMA and ionomycin (Figure 2, Lane 6). It is noteworthy that higher concentrations of PatA or DMDAPatA did not affect the transcription of PGK which would indicate general inhibition of cell proliferation or induction of apoptosis, strongly suggesting that the effects of both PatA and DMDAPatA on IL-2 is due to specific inhibition of signaling leading to IL-2 promoter activation. Together, the results with DMDAPatA support the hypothesis that the C1−C5 segment of PatA does not interact directly with its putative cellular receptor leading to inhibition of IL-2 transcription but that this sector of the molecule may simply serve as a scaffold to define and maintain the macrocyclic conformation. Importantly, this derivative was also more stable than PatA (stable in $CDCl_3$ for 3−4 weeks at 25 °C; PatA decomposes in $CDCl_3$ at 25 °C in <10 min).

Conclusion

In summary, a hypothesis was developed regarding a potential binding and scaffolding domain in the immunosuppressive and anticancer marine natural product, pateamine A. This hypothesis was premised on preliminary biological studies, structural analysis, and simple molecular modeling studies of PatA and derivatives. To lend support to this hypothesis, the simplified derivative DMDAPatA **3** devoid of the C3-amino and C5-methyl groups was designed, synthesized, and found to have similar to greater potency than PatA in the IL-2 reporter gene assay. This result provides convincing evidence for the hypothesis that this sector of the molecule (C1−C5) merely serves as a scaffold for the remaining conformationally rigid sectors (C6−C24) of the molecule including the thiazole, the dienoate, and the triene side chain. These findings are reminiscent of similar receptor binding proposals put forth in early studies of cyclic peptide and macrocyclic immunosuppressive natural products, namely cyclosporin A, FK506, and rapamycin.[2] However, in those cases, the domains were renamed effector and binding domains due to the fact that these natural products were found to bind two cellular proteins acting as molecular "glue." Importantly, the

synthesis of this derivative (14 versus 24 steps from crotyl alcohol) is greatly simplified relative to PatA (**1**) being devoid of two stereocenters and having greater stability.

As previously observed, C3-amino acylated derivatives of PatA retain activity in the IL-2 reporter gene assay. In addition, a subtle interplay between the dienoate sector (C18−C22) and the triene side chain was revealed when dienoate versus enynoate-containg macrocycles were compared. This appears to suggest the importance of macrocycle conformation and side chain functionality in binding of PatA to its putative cellular receptor. Current studies are directed toward characterizing the cellular protein receptor(s) of PatA and the results of these studies will be reported in due course.

Experimental Section

Modified Nagao Acetate Aldol: β-Hydroxy Amide 6. To a cold (−40 °C) stirred solution of thiazolidine thione **4** (6.59 g, 32.50 mmol) in dry CH_2Cl_2 (115 mL) was added $TiCl_4$ (35 mL, 1 M in CH_2Cl_2, 35.0 mmol). The resulting solution was stirred for 5 min at −40 °C, then *i*-Pr_2NEt (6.0 mL) was added. The resulting black slurry was vigorously stirred for 2 h at −40 → 60 °C. Then a solution of aldehyde **5** (4.14 g, 27.89 mmol) in CH_2Cl_2 (5 mL) was added to the mixture at −78 °C. The mixture was stirred at −78 °C for 15 min and then 20 mL of pH 7 buffer was added to quench the reaction. The aqueous layer was extracted with EtOAc, the combined organic layer was washed with brine, dried over $MgSO_4$. Concentration and purification by flash chromatography eluting with EtOAc:hexanes (1.5) gave aldol adduct **6** (7.81 g, 80%) as a yellow oil with spectral and physical data matching that previously reported and the remaining steps leading to thioamide **7** were performed as previously described.[11]

Trichloroethyl Ester 9. To a stirred solution of 6-oxoheptanoic acid (2.0 g, 12.58 mmol) in benzene (40 mL) was added trichloroethanol (1.08 mL, 11.32 mmol) and $SOCl_2$ (1.1 mL, 15.1 mmol). The solution was refluxed for 8 h and then evaporated and diluted with 30 mL of EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash column chromatography on SiO_2 eluting with EtOAc:hexanes (1:4) gave 2.1 g (61%) of ester **5** as a light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71(s, 2H), 2.46−2.42 (m, 4H), 2.11 (s, 3H), 1.65−1.62 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.4, 171.8, 95.6, 74.1, 43.2, 33.8, 30.1, 24.3, 23.2; IR (neat) 2956, 1762, 1720 cm^{-1}.

α-Bromoketo Ester 10. To a cooled (0 °C) stirred solution of ester **9** (5.91 g, 21.5 mmol) in dry CH_2Cl_2 (350 mL) was added DIPEA (18.5 mL, 0.106 mol) and TMSOTf (15.5 mL, 85.74 mmol). Stirring was continued at 0 °C for 1.5 h. The reaction was quenched with aqueous $NaHCO_3$ (70 mL). The aqueous layer was extracted with hexane, the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was used in the next step without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (s, 2H), 4.04 (s, 2H), 2.48 (t, 2H, J = 8.2 Hz), 2.04 (t, 2H, J = 8.2 Hz), 1.78−1.64 (m, 2H), 1.59−1.50 (m, 2H).

To a cooled (−78 °C) stirred solution of the crude TMS enol ether (8.1 g) in dry THF (350 mL) was added 2.2 g $NaHCO_3$ and NBS (4.0 g, 22.5 mmol). The resulting mixture was stirred at −78 °C for 2 h and then the reaction was quenched with aq. $NaHCO_3$ (100 mL). The aqueous layer was extracted with hexane, the combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue was accomplished by directly loading onto a flash column with SiO_2 eluting with EtOAc:hexanes (25:1→10:1) to give 6.04 g (79% for two steps) of bromoketo ester **10** as a pale yellow oil:^{1}H NMR (300 MHz, $CDCl_3$) δ 4.75(s, 2H), 3.88 (s, 2H), 2.74−2.68 (m, 2H), 2.56−2.44 (m, 2H), 1.74−1.66 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.7, 171.7, 95.1, 74.0, 39.4, 34.3, 33.7, 24.1, 23.2; IR (neat) 2934, 1756, 1710 cm^{-1}; HRMS (ESI) Calcd for $C_9H_{12}Br\ Cl_3O_3$ [M+H]: 376.8903 Found: 376.8901.

Thiazole 12. To a cooled (−5 °C), stirred solution of α-bromoketo ester **10** (4.50 g, 12.7 mmol) in CH_2Cl_2 (250 mL) was added 2,6-lutidine (2.80 mL, 24.1 mmol) and thioamide **7**[11] (3.72 g, 9.79 mmol). The solution was stirred at 25 °C for 18 h. The reaction mixture was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Rapid purification of the residue by flash column chromatography on SiO_2 eluting with EtOAc:hexanes (1:4) gave 4.06 g (64%) of the intermediate thiazoline **11** as a colorless oil and as a mixture of diastereomers which was used directly in the next step. Partial spectral data is provided: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.91 (dt, J = 1.8, 9 Hz, 1H), 4.82−4.77 (m, 1H), 4.77(s, 2H), 3.29 (s, 2H), 2.91−2.81 (m, 2H), 2.72 (ddd, J = 7.2, 8.7, 14.1, 1H), 2.53 (t, J = 7.5 Hz, 2H), 2.29 (s, 2H), 1.89 (t, J = 9.3 Hz, 2H), 1.78 (t, J = 7.2 Hz, 2H), 1.63−1.48 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 134.9, 108.5, 74.1, 68.9, 68.6, 43.6, 43.5, 43.4, 43.2, 40.9, 34.1, 25.2, 24.7, 23.7, 18.3, 18.1, 12.5. To a cooled (0 °C), stirred solution of thiazoline **11** (3.46 g, 5.29 mmol) in CH_2Cl_2 (70 mL) was added Hünig's base (9.63 mL, 55.4 mmol), pyridine (1.53 mL, 18.9 mmol) and TFAA (2.61 mL, 18.5 mmol) and the solution was stirred at 25 °C for 3 h and then diluted with 50 mL of CH_2Cl_2. The organic layer was washed with satd. aqueous $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash column chromatography on SiO_2 eluting with EtOAc:hexanes (1:4) gave 3.26 g (97%) of thiazole **12** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78 (s, 1H), 5.89 (dd, J = 0.6, 8.7 Hz, 1H), 4.80 (dt, J = 6.0, 9.0 Hz, 1H), 4.76 (s, 2H), 3.25 (dd, J = 6.3, 14.1 Hz, 1H), 3.13 (dd, J = 6.3, 14.1 Hz, 1H), 2.80−2.75 (m, 2H), 2.54−2.50 (m, 2H), 2.11 (s, 3H), 1.85−1.72 (m, 4H), 1.04 (s, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 165.4, 156.6, 135.1, 121.5, 113.4, 95.2, 74.1, 70.3, 42.2, 33.9, 31.3, 28.8, 24.5, 24.2, 18.2, 12.5; HRMS (ESI) Calcd for $C_{24}H_{40}BrCl_3NO_3SSi$ [M+H]: 634.0717 Found: 634.0748.

Thiazole Enyne 15. To a cooled (−20 °C), stirred solution of thiazole **12** (3.17 g, 4.99 mmol) in THF (50 mL) was added 15 mL of 1M TBAF (15.0 mmol) buffered with 20 mol % AcOH and the solution was stirred at −20 °C for 3 h. The reaction mixture was diluted with 50 mL of CH_2Cl_2. The organic layer was washed with satd. aq. $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. Coarse purification of the residue by flash column chromatography on SiO_2 eluting with EtOAc:hexanes (1:4→1:1) gave 38 mg of alcohol **13** (2.30 g, 97%) as a light yellow oil which was used directly in the next step. To a solution of DIAD (0.91 mL, 4.63 mmol) in THF (30 mL) was added PPh_3 (0.97 g, 3.70 mmol) as a solid and the solution was stirred at ambient temperature for 30 min. The resulting heterogeneous mixture was cooled (−20 °C) and the solution of acid **14** (0.60 g, 1.35 mmol) in THF (0.2 mL) was added. After 20 min, a solution of alcohol **13** (0.39 g, 0.81 mmol) in THF (4.0 mL) was added and stirring was continued for 1 h at −20 → -30 °C. The reaction was quenched by addition of 10 mL pH 7 buffer followed by warming to 25 °C and diluting with 100 mL of EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by flash column chromatography on SiO_2 eluting with EtOAc:hexanes (1:15) gave 580 mg (91%) of thiazole enyne **15** as a light yellow oil:^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (s, 1H), 5.87 (dd, J = 1.2, 9.6 Hz, 1H), 5.79−5.72 (m, 1H), 5.38 (d, J = 1.2 Hz, 1H), 4.71 (s, 2H), 4.13−4.07 (m, 1H), 3.36 (dd, J = 6.9, 14.7 Hz, 1H), 3.24 (dd, J = 6.9, 14.7 Hz, 1H), 2.74 (t, J = 6.9 Hz, 1H), 2.48 (t, J = 6.9 Hz, 2H), 2.39 (dd, J = 5.4, 14.7 Hz, 1H), 2.23 (dd, J = 5.4, 14.7 Hz, 1H), 2.26 (d, J = 1.2 Hz, 1H), 1.99 (d, J = 1.5 Hz, 3H), 1.77−1.69 (m, 4H), 1.24−1.22 (m, 2H), 1.11 (d, J = 6.3 Hz, 3H), 1.03 (s, 21H); HRMS (ESI) Calcd for $C_{33}H_{50}Br\ Cl_3NO_5SSi$ [M+H]: 784.1428 Found: 784.1434.

Hydroxy Ester 16. To a stirred solution of silyl ether **15** (0.564 g, 0.72 mmol) in THF (10 mL) was added a 50% AcOH/TBAF solution (3.60 mL, 3.60 mmol). The resulting solution was stirred at 25 °C for

8 h. The reaction mixture was diluted with 50 mL of CH_2Cl_2. The organic layer was washed with $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue was accomplished by directly loading onto a flash column chromatography of SiO_2 eluting with EtOAc:hexanes (1:1) gave 362 mg (80%) of alcohol **16** as a yellowish oil:^{1}H NMR (500 MHz, $CDCl_3$) δ 6.84 (s, 1H), 5.92 (dd, 1H, J = 1.2, 6.9 Hz), 5.86−5.79 (m, 1H), 5.48 (s, 1H), 4.77 (s, 2H), 4.07−4.01 (m, 1H), 3.43 (dd, J = 6.9, 14.7 Hz, 1H), 3.29 (dd, J = 6.9, 14.7 Hz, 1H), 2.81 (t, 2H, J = 6.6 Hz), 2.54 (t, 1H, J = 6.9 Hz), 2.32 (d, 3H, J = 1.5 Hz), 2.07 (d, 3H, J = 1.2 Hz), 1.80−1.76 (m, 4H), 1.26 (d, 3H, J = 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) d 172.1, 171.4, 163.7, 158.5, 156.9, 153.2, 128.6, 128.1, 113.8, 105.2, 85.6, 83.7, 74.1, 71.6, 65.9, 48.9, 38.0, 33.9, 31.2, 28.7, 24.5, 23.6, 20.7, 14.4; HRMS (ESI) Calcd for $C_{24}H_{39}Br\ Cl_3NO_5S$ [M+H]: 628.0094 Found: 628.0073

Macrocycle 18. To a stirred solution of alcohol **16** (342 mg, 0.54 mmol) in THF (7.5 mL) and 1M NH_4OAc (7.5 mL) was added 10% Cd/Pd couple (500 mg) The resulting solution was stirred at 25 °C for 8 h. The reaction mixture was diluted with 10 mL of EtOAc and then filtered through a pad of diatomaceous earth. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromotagraphy on SiO_2 eluting with EtOAc: MeOH (10.1) gave hydroxy acid **17** as a colorless oil (240 mg, 89%).

To a cooled (0 °C) stirred solution of hydroxy acid **17** (62.0 mg, 0.12 mmol) in THF (3.0 mL) was added Et_3N (110 μL, 0.72 mmol) and 2,4,6-trichlorobenzoyl chloride (98 μL, 0.63 mmol). The resulting solution was stirred at 0 °C for 20 min and then added to a solution of DMAP (152 mg, 1.26 mmol) in toluene (62 mL) at 25 °C and stirred for 2 h. The reaction mixture was diluted with 50 mL of EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash column chromatography on SiO_2 eluting with EtOAc:hexanes (1:3) gave 50.0 mg (84%) of macrocycle **18** as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 (s, 1H), 6.06 (dd, J = 1.2, 9.3 Hz, 1H), 5.93−5.86 (m, 1H), 5.35 (s, 1H), 5.33−5.26 (m, 1H), 3.34 (br d, J = 7.5 Hz, 2H), 2.81−2.61 (m, 2H), 2.46−2.39 (m, 1H), 2.43 (d, J = 1.5 Hz, 3H), 2.30 (d, J = 6.9 Hz, 2H), 1.96 (d, J = 1.2 Hz, 3H), 1.90−1.68 (m, 3H), 1.57−1.45 (m, 3H), 1.28 (d, J = 6.3 Hz, 3H); HRMS (ESI) Calcd for $C_{22}H_{27}Br\ Cl_3NO_4S$ [M+H]: 480.0844 Found: 480.0760.

DMDA PatA (3). A slurry of $Pd/CaCO_3$ poisoned with Pb (52 mg) and macrocycle **18** (117 mg, 0.24 mmol) in 10 mL of MeOH was evacuated under water aspirator pressure and purged with H_2. After stirring at 25 °C for 3 h under 1 atm of H_2, the reaction was filtered through diatomaceous earth and concentrated in vacuo. Passage through a plug of SiO_2 eluting with EtOAc:hexanes (50:50) gave 66 mg (56%) of *E*,*Z*-macrocycle **19** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (d, J = 11.7 Hz, 1H), 6.72 (s, 1H), 6.71 (dd, J = 11.7 Hz, 1H), 6.08 (dt, J = 4.5, 16.5 Hz, 1H), 5.97 (dq, J = 1.2, 9.6 Hz, 1H), 5.36 (d, J = 11.7 Hz, 1H), 5.23−5.12 (m, 1H), 3.27−3.14 (m, 2H), 2.93−2.83 (m, 1H), 2.60 (ddd, J = 4.5, 10.5, 1.4 Hz, 1H), 2.53−2.47 (m, 1H), 2.51 (s, 3H), 2.41−2.13 (m, 4H), 1.86 (s, 3H), 1.77−1.61 (m, 2H), 1.44−1.29 (m, 2H), 1.27 (d, J = 6.6 Hz, 3H). This material was directly used in the next reaction without further purification. To a flask charged with $Pd_2dba_3{\cdot}CHCl_3$ (17 mg, 0.016 mmol) and triphenyl arsine (41 mg, 0.13 mmol) was added 1.0 mL of degassed THF prepared by several freeze/thaw cycles. The final concentration of this palladium catalyst stock solution was ~0.031 M. To a solution of macrocycle **19** (66 mg, 0.14 mmol) and stannane **20**[11] (113 mg, 0.27 mmol) in 4.0 mL of THF was added 450 μL (0.014 mmol, 10 mol %) of palladium catalyst stock solution. After stirring the resulting homogeneous solution at 25 °C for 4 h, another 300 μL of palladium catalyst stock solution was added and stirring was continued for an additional 12 h. Concentration in vacuo and purification of the residue by flash column chromatography on SiO_2 eluting with EtOAc:Et_3N (20:1) gave 54 mg (76%) of DMDAPatA (**3**) as a pale yellow oil: ^{1}H NMR (500 MHz, C_6D_6) δ 7.47 (d, J = 12.0 Hz, 1H), 6.71 (app dt, J = 5.0, 9.0 Hz, 1H), 6.45 (app t, J = 11.5 Hz, 1H), 6.32 (d, J = 16.0 Hz, 1H), 6.21 (d, J = 16.0 Hz, 1H), 6.17 (s, 1H), 5.69 (t, J = 7 Hz, 1H), 5.55 (d, J = 11.5 Hz, 1H), 5.49 (d, J = 9.0 Hz, 1H), 5.19−5.11 (m, 1H), 3.08−3.01 (m, 2H), 2.91 (d, J = 6.5 Hz, 2H), 2.78 (dt, J = 4.5, 14.0 Hz, 1H), 2.48−2.42 (m, 1H), 2.33 (ddd, J = 4.0, 10.0, 14.5 Hz, 1H), 2.11 (s, 6H), 2.16−2.03 (m, 2H), 1.90 (d, J = 1.0 Hz, 3H), 1.71 (2, 3H), 1.64−1.61 (m, 1H), 1.56−1.43 (m, 2H), 1.54 (s, 3H), 0.96−0.81 (m, 2H), 0.95 (d, J = 6.5 Hz, 3H); HRMS (ESI) Calcd for $C_{30}H_{43}N_2O_4S$ [M+H]: 527.2944 Found: 527.2927.

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Supporting Information Available: General procedures for synthesis of PatA derivatives **26**−**32** and **35**−**36** and ^{1}H NMR spectra for compounds **3**, **9**−**10, 11**−**13**, **15**−**16**, **18**−**19**, **26**−**32**, and **35**−**36**. This material is available free of charge via the Internet at http://www.acs.pubs.org.

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