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Enantiomeric Discorhabdin Alkaloids and Establishment of Their Absolute Configurations Using Theoretical Calculations of Electronic Circular Dichroism Spectra

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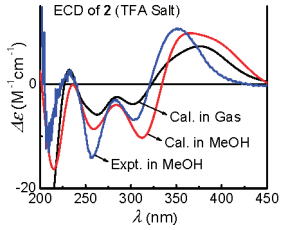
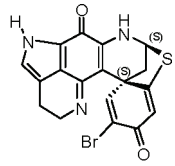
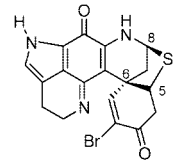
Received July 22, 2008



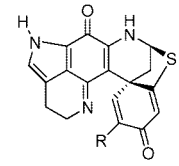


Enantiomeric pairs of the cytotoxic pyrroloiminoquinone marine alkaloids discorhabdins B (**2**), G*/I (**3**), L (**4**), and W (**5**) have been isolated from *Latrunculia* species sponges collected at different locations around the coast of New Zealand. The absolute configuration of all compounds was secured by comparison of observed data with the results of time-dependent density functional theory (TDDFT) calculations of electronic circular dichroism (ECD) spectra. Enantiomeric discorhabdins exhibit equipotent antiproliferative biological activity.

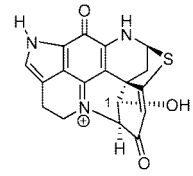
The discorhabdins/prianosins are a structurally diverse family of cytotoxic alkaloids bearing a common pyrroloiminoquinone core that are isolated from marine sponges of the family Latrunculiidae and Acarnidae.[1,2] A New Zealand collection of the sponge *Latrunculia brevis*[3] yielded the first alkaloid in the series, discorhabdin C,[4] while the first chiral example, prianosin (discorhabdin) A [(+)-**1**] was reported from both Japanese and New Zealand sponges.[5,6] For both alkaloids X-ray crystal analysis was pivotal in structure determination, and in the case of (+)-**1**, assignment of absolute configuration as (5*R*,6*S*,8*S*).[5] Since then, a number of related compounds were reported from collections of Latrunculiidae sponges undertaken in geographically diverse regions including Japan, New Zealand, Fiji, Australia, South Africa, South America, and Antarctica.[1] In all of these subsequent cases, no effort has been made to relate any observed chiroptical properties to alkaloid absolute configuration. As part of an ongoing study of the structure−activity relationship of the discorhabdin alkaloids[7] we required further quantities of discorhabdin B (**2**) for which a survey of a number of different sponge specimens collected from around the New Zealand coast was undertaken (for the current taxonomy of the New Zealand species and collection locations see the Supporting Information).[3] While our investigation of Wellington-sourced *Latrunculia* sp. afforded discorhabdins B (**2**), G*/I (**3**), L (**4**), and W (**5**), specimens of *Latrunculia* sp. collected in the geographically remote region of New Zealand's west coast at Doubtful and Milford Sounds yielded the same alkaloids that exhibited equal and opposite specific rotation and electronic circular dichroism (ECD) spectra. In this Note we report the structural and chiroptical characterization, including time-dependent density functional theory calculations of ECD spectra that led to the determination of absolute configuration of the first enantiomeric series of discorhabdin alkaloids.



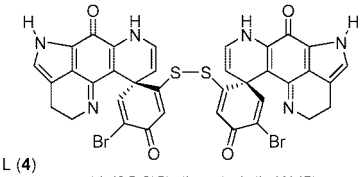
(+)-(5*R*,6*S*,8*S*)-discorhabdin A (**1**)



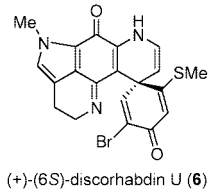
(+)-(6*S*,8*S*)-discorhabdin B (**2**) R = Br
(+)-(6*S*,8*S*)-discorhabdin G*/I (**3**) R = H



(-)-(1*R*,2*S*,6*R*,8*S*)-discorhabdin L (**4**)



(-)-(6*S*,6′*S*)-discorhabdin W (**5**)



(+)-(6*S*)-discorhabdin U (**6**)

Discorhabdin B isolated from Wellington *Latrunculia* sp. exhibited $[\alpha]_D$ values of the same sign and similar magnitude

[†] The University of Auckland.
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(1) Antunes, E. M.; Copp, B. R.; Davies-Coleman, M. T.; Samaai, T. *Nat. Prod. Rep.* **2005**, *22*, 62–72.
(2) Lang, G.; Pinkert, A.; Blunt, J. W.; Munro, M. H. G. *J. Nat. Prod.* **2005**, *68*, 1796–1798.
(3) Two more recent taxonomic revisions of *Latrunculia* have been reported: (a) Alvarez, B.; Bergquist, P. R.; Battershill, C. N. *N. Z. J. Mar. Freshwater Res.* **2002**, *36*, 151–184. (b) Samaai, T.; Gibbons, M. J.; Kelly, M. *Zootaxa* **2006**, *1127*, 1–71.
(4) Perry, N. B.; Blunt, J. W.; McCombs, J. D.; Munro, M. H. G. *J. Org. Chem.* **1986**, *51*, 5476–5478.
(5) Kobayashi, J.; Cheng, J.-F.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Lu, H.; Clardy, J. *Tetrahedron Lett.* **1987**, *28*, 4939–4942.

10.1021/jo801622n CCC: $40.75 © 2008 American Chemical Society
Published on Web 10/15/2008

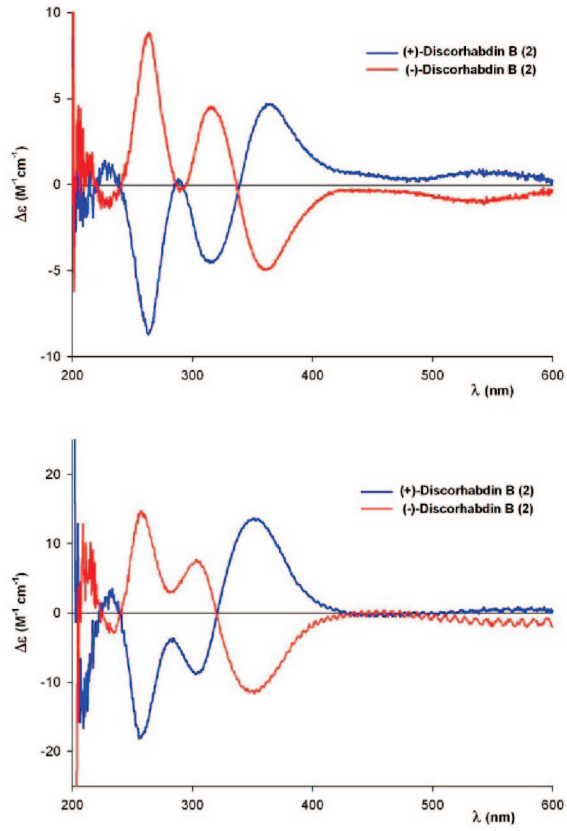

FIGURE 1. Experimental ECD spectra of (+)- and (−)-enantiomers of discorhabdin B (**2**) as free base (upper) and trifluoroacetate salt (lower).

(+320 for trifluoroacetate salt) to that originally reported (+400 for HCl salt).[6] Sponges, however, collected from Doubtful and Milford Sounds on the South Western coast of New Zealand consistently yielded (−)-**2** ($[\alpha]_D$ −400 for TFA salt), which as either the trifluoroacetate salt or free base also exhibited mirror image ECD spectra versus material isolated from Wellington sponges (Figure 1).

The enantiomeric nature of (+)-**2** and (−)-**2** was further investigated by using cyclodextrins (CDX) as chiral NMR shift reagents.[8] Titrations of a racemic mixture of **2**, prepared by mixing enantiomers of **2** until $[\alpha]_D = 0$, with either of α-, *β*-, or *γ*-CDX failed to induce any relative shift in observed ^{1}H NMR resonances.

β-CDX titrations with either of (+)- or (−)-discorhabdin U, (+)/(−)-(**6**), prepared by reaction of (+)/(−)-**2** with trimethylphosphate or methyl iodide and K_2CO_3,[7] however, did result in detectable shifts of H-1, H-4, H-16, and *S*Me of **6**. Maximal shifts were observed when the ratio of *β*-CDX to **6** reached 3:1. Host−guest binding was confirmed by the observation of ROESY NMR (τ_{mix} 800 ms) correlations between H-3′ and H-5′ of *β*-CDX and H-1, H-4, and H-16 (weak) and *S*Me of **6**. Addition of *β*-CDX to *rac*-**6**, prepared by combining both enantiomers of **6**, afforded ^{1}H NMR spectra that revealed doubling of resonances[9] consistent with the formation of diastereomeric host−guest complexes (see the Supporting Information). The observation of a single set of resonances for both *β*-CDX and each of (+)- and (−)-**6** led to the conclusion that discorhabdin B (**2**) isolated from sponges was >90% enantiopure.

The absolute configuration of a chiral molecule can be determined by using time-dependent density functional theory

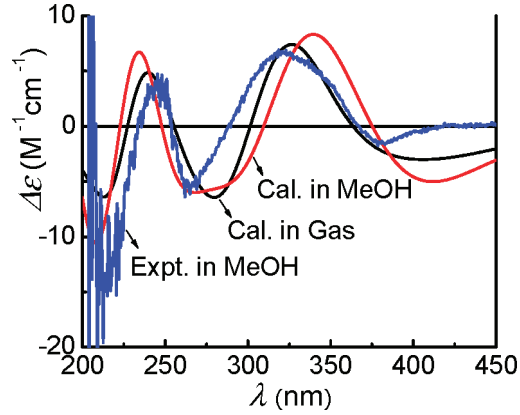

FIGURE 2. Calculated ECD spectra (black line, at the B3LYP/6-31G** level; red line, at the B3LYP-SCRF/6-31G** level; $\sigma = 0.20$ eV) for (+)-(5*R*,6*S*,8*S*)-discorhabdin A (**1**) in the gas phase (black) and the methanol solvation model (red) compared to the experimental spectrum observed for (+)-**1** (blue) as free base.

(TDDFT) calculations of ECD spectra.[10] This technique is particularly attractive in the case of the discorhabdins due to their rigid, conformationally restrained structures and strong optical rotatory and circular dichroism properties. To explore the feasibility and reliability of the method on this class of compounds, we first undertook ECD calculations for (+)-(5*R*,6*S*,8*S*)-discorhabdin/prianosin A (**1**), the absolute configuration of which was previously defined by X-ray crystal analysis.[5] The geometry of **1** was based on its crystal structure and optimized at the B3LYP/6-31G** level, affording a single optimized conformer. The ECD calculations of **1** were then conducted at 298 K in the gas phase at the B3LYP/6-31G** level and further in MeOH at the B3LYP-SCRF/6-31G**// B3LYP/6-31G** level. The calculated and experimentally observed ECD spectra are in good agreement (Figure 2).

With confidence in the DFT method using appropriate functional and basis sets, we applied it to (6*S*,8*S*)-**2**, performing the ECD calculations for both the free base and the trifluoroacetate salt forms at the same level. Comparison of experimental and theoretically simulated ECD curves of both the trifluoroacetate salt and free base (Figure 3) forms of (6*S*,8*S*)-**2** unambiguously demonstrates that (+)-**2** has the (6*S*,8*S*) configuration.

The finding that disparate collections of New Zealand *Latrunculia* species sponges can yield antipodal discorhabdin B prompted us to undertake further comparative analysis of these extracts. Both sponge populations led to the isolation of known discorhabdins G*/I (**3**), L (**4**), and W (**5**).[2,11,12] Discorhabdin (+)-W [(+)-**5**], the first example of a dimeric discorhabdin alkaloid, was recently reported by Lang et al. from a Milford

(6) Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron* **1988**, *44*, 1727–1734.

(7) Grkovic, T.; Kaur, B.; Webb, V. L.; Copp, B. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1944–1946. An alternative route to discorhabdin U from discorhabdin B is to utilize methylation with trimethylphosphate in the presence of excess K_2CO_3 (70% yield).

(8) Pearce, A. N.; Babcock, R. C.; Battershill, C. N.; Lambert, G.; Copp, B. R. *J. Org. Chem.* **2001**, *66*, 8257–8259.

(9) The H-8 and H-14 resonances did not shift, while signals for H-17 and *N*-Me of **6** were obscured by *β*-CDX or HOD signals.

(10) (a) Diedrich, C.; Grimme, S. *J. Phys. Chem. A* **2003**, *107*, 2524–2539. (b) Crawford, T. D.; Tam, M. C.; Abrams, M. L. *J. Phys. Chem. A* **2007**, *111*, 12057–12068. (c) Stephens, P. J.; Devlin, F. J.; Gasparrini, F.; Ciogli, A.; Spinelli, D.; Cosimelli, B. *J. Org. Chem.* **2007**, *72*, 4707–4715. (d) Ding, Y.; Li, X.-C.; Ferreira, D. *J. Org. Chem.* **2007**, *72*, 9010–9017. (e) Berova, N.; Bari, L. D.; Pescitelli, G. *Chem. Soc. Rev.* **2007**, *36*, 914–931.

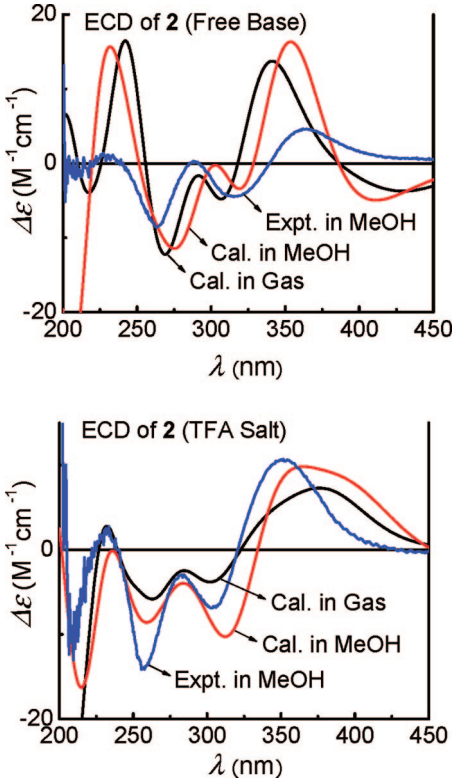

FIGURE 3. Calculated ECD spectra (black line, at the B3LYP/6-31G** level; red line, at the B3LYP-SCRF/6-31G** level) for (6*S*,8*S*)-discorhabdin B (**2**) in the gas phase (black) and the methanol solvation model (red) compared to the experimental spectrum observed for (+)-**2** (blue) as either free base (upper) or trifluoroacetate salt (lower).

Sound, New Zealand-sourced sponge.[2] Our natural product isolated from sponges collected in the same locale exhibited identical specific rotation properties to those reported (free base $[\alpha]_D$ +240, lit.[2] $[\alpha]_D$ +220), while Wellington Harbor-sourced sponge yielded enantiomeric (−)-**5** ($[\alpha]_D$ −260) (see the Supporting Information). Gas phase calculation of the ECD spectrum of the free base (6*S*,6′*S*) configuration of discorhabdin W (**5**) using the predominant conformer (96% population, see the Supporting Information) showed good agreement with the spectrum observed for the alkaloid isolated from the Wellington *Latrunculia* sp., thereby establishing that (−)-discorhabdin W has the (6*S*,6′*S*) configuration (see the Supporting Information).[13] In agreement with the previously reported analytical-scale reactions,[2] reduction of (−)-discorhabdin W (**5**) with TCEP yielded (+)-discorhabdin B (**2**), and exposure to light promoted the dimerization of (+)-discorhabdin B (**2**) to (−)-W (**5**). Both semisynthetic discorhabdins B and W were identical in all aspects to the naturally occurring compounds, thereby conclusively proving the absolute configuration of (−)-discorhabdin W as (6*S*,6′*S*).

The two enantiomers of discorhabdin G*/I (**3**), isolated from Wellington Harbor- and Doubtful Sound-sourced collections of *Latrunculia* spp. exhibited opposite specific rotation data, ($[\alpha]_D$ +540, $[\alpha]_D$ −720, respectively; lit.[12] $[\alpha]_D$ −563), and equal and opposite ECD spectra (see the Supporting Information). The experimental ECD spectrum of (+)-discorhabdin G*/I ((+)-**3**) (trifluoroacetate salt), which was essentially identical to that observed for (+)-(6*S*,8*S*)-discorhabdin B trifluoroacetate salt [(+)-**2**], had a high level of agreement with that calculated by TDDFT methods thereby demonstrating that (+)-discorhabdin G*/I [(+)-**3**] has the (6*S*,8*S*) configuration (see the Supporting Information).

The original report of the isolation of discorhabdin L (1*R**-hydroxydiscorhabdin D) (**4**), from a South American collection of *Latrunculia brevis*, noted that despite containing four stereogenic centers, the alkaloid exhibited an $[\alpha]_D$ value of zero.[12] Samples of discorhabdin L isolated from both Wellington and Doubtful Sound *Latrunculia* spp. collections exhibited identical ^{1}H, ^{13}C, and NOE NMR spectroscopic and mass spectrometric properties to those previously reported. Both samples of **4** also failed to exhibit optical rotation at the sodium D-line but were optically active at other wavelengths[14] and yielded equal and opposite ECD spectra (see the Supporting Information). The experimental ECD spectrum of Wellington Harbor-sourced alkaloid [$(-)_{578}$-**4**] had a high level of agreement with the spectrum calculated by TDDFT methods for the (1*R*,2*S*,6*R*,8*S*)-configuration (see the Supporting Information).

The isolation of enantiomers of marine natural products from source organisms collected at different locations has been previously reported.[15] To put our discovery of discorhabdin enantiomers in a global perspective a number of *Latrunculia* sponge specimens were screened for the presence of specific enantiomers of **2**−**5** from a diverse range of locations around the coast of New Zealand (see the Supporting Information) as well as Antarctica and South Africa. The Milford Sound/Doubtful Sound region was found to be the unique New Zealand locale for the presence of (−)-discorhabdin B, (+)-discorhabdin W, (−)-discorhabdin G*/I, and (+)-discorhabdin L. A South African-sourced sponge extract also yielded (−)-G*/I and (+)-L.[16] An Antarctic specimen of *Latrunculia* sp. afforded (+)-discorhabdin B and (−)-discorhabdin W. Somewhat anomalously a South American collection of *Latrunculia brevis* that yielded (−)-(6*R*,8*R*)-discorhabdin G*/I[12] also contained the enantiomeric scaffold (+)-(6*S*,8*S*)-discorhabdin B.[18] It is interesting to note that in all cases the alkaloids were isolated in optically active form and that no occurrences of biosynthesis producing racemic mixtures was detected. While comprised of a very limited data set, the current study highlights the enantiomeric specificity in the biosynthesis of thioether-containing discorhabdin alkaloids and that variation in absolute configuration occurs with location.

(11) Antunes, E. M.; Beukes, D. R.; Kelly, M.; Samaai, T.; Barrows, L. R.; Marshall, K. M.; Sincich, C.; Davies-Coleman, M. T. *J. Nat. Prod.* **2004**, *67*, 1268–1276.

(12) Reyes, F.; Martín, R.; Rueda, A.; Fernández, R.; Montalvo, D.; Gómez, C.; Sánchez-Puelles, J. M. *J. Nat. Prod.* **2004**, *67*, 463–465.

(13) Only the ECD in the gas phase was calculated due to limitations of computational time for such a conformationally mobile bulky molecule.

(14) Wellington-sourced **4** exhibited specific rotation values of $[\alpha]_{578}$ =−145, $[\alpha]_{546}$ =−185, and the Doubtful Sound-sourced **4** $[\alpha]_{578}$ = +200, $[\alpha]_{546}$ = +500 (*c* 0.05, MeOH).

(15) Examples of marine natural product enantiomers include: (a) Reference 8. (b) Copp, B. R.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *Tetrahedron Lett.* **1989**, *30*, 3703–3706. (c) Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R. M.; Tsukamoto, S. *Angew. Chem.* **2007**, *46*, 2254–2256. (d) Greshock, T. J.; Grubbs, A. W.; Jiao, P.; Wicklow, D. T.; Gloer, J. B.; Williams, R. M. *Angew. Chem.* **2008**, *47*, 3573–3577. (e) Tsuda, M.; Kawasaki, N.; Kobayashi, J. *Tetrahedron* **1994**, *50*, 7957–7960. (f) Fontana, A.; Fakhr, I.; Mollo, E.; Cimino, G. *Tetrahedron: Asymmetry* **1999**, *10*, 3869–3872. (g) Gregson, R. P.; Ouvrier, D. J. *J. Nat. Prod.* **1982**, *45*, 412–414. (h) Uenishi, J.; Kawahama, R.; Yonemitsu, O. *J. Org. Chem.* **1997**, *62*, 1691–1701.

(16) We gratefully acknowledge Professor Mike Davies-Coleman (Rhodes University, South Africa) for supplying a sample of crude extract of South African-sourced *Cyclanthia bellae*.[17]

(17) Samaai, T.; Govender, V.; Kelly, M. *Zootaxa* **2004**, *725*, 1–18.

(18) We are also grateful to Dr. Fernando Reyes (PharmaMar, Spain) for supplying samples of discorhabdins A and B isolated in their study of a South American specimen of *Latrunculia brevis*.[12]

Discorhabdin/prianosin alkaloids are noted for their potent in vitro cytotoxicity, the mechanism(s) of action of which are currently undefined. The enantiomeric pairs of discorhabdins B, W, G*/I, and L were found to exhibit similar cytotoxic potency toward the P388 murine leukemia cell line with IC_{50} values of 0.2 μM and 0.17 μM [for (+)- and (−)-**2**, respectively], 0.6 μM/0.53 μM [for (+)- and (−)-**3**], 0.78 μM/1.08 μM [for (+)- and (−)-**4**], and 0.1 μM/0.13 μM [for (+)- and (−)-**5**]. These results support the observation of Kita et al. that enantiomers of discorhabdin A were also equipotent toward the HCT-116 cell line.[19]

In summary, we have identified for the first time enantiomeric pairs of discorhabdin alkaloids from New Zealand collections of *Latrunculia* spp. sponges. The finding that specific populations of sponge can biosynthesize enantiomers in this series emphasizes the importance in obtaining chiroptical properties when such alkaloids are characterized. Excellent agreement was observed between experimental electronic circular dichroism spectra and those calculated by using TDDFT allowing the absolute configuration of all alkaloids to be determined. These results will facilitate assignment of absolute configuration of discorhabdin alkaloids reported in the future.

Experimental Section

General Experimental Procedures. ECD spectra were recorded on an Applied Photophysics Pi star spectrophotometer. All other general experimental procedures have been described elsewhere.[20]

Collection, Extraction, and Isolation Procedures. The specimens of *Latrunculia* spp. sponges collected in New Zealand and Antarctic waters were from the NIWA marine organism collection. Taxonomic identification of these species has been previously described.[3] For details of the metabolite isolation procedures and compound characterization, see the Supporting Information.

Computational Chemistry. For details of DFT calculations, using Gaussian 03,[21] see the Supporting Information.

Acknowledgment. The authors thank Professor T. Brittain (UoA) for access to the ECD spectrophotometer, Mr. M. Walker and Dr. M. Schmitz for assistance with NMR data acquisition, and Ms. R. Imatdieva (UoA) and Ms. P. Greed (University of Waikato) for MS data. P388 assays were run by Ms. G. Ellis (University of Canterbury). T.G. acknowledges the University of Auckland for a UoA Doctoral Scholarship. We also gratefully acknowledge the collegiality of Professor M. Davies-Coleman and Dr. F. Reyes for kindly providing an extract of South African *Cyclanthia bellae* or pure samples of alkaloids isolated from South American *Latrunculia brevis* respectively for inclusion in this study. This work was supported by the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-2-0009.

Supporting Information Available: Isolation details, spectroscopic data (NMR, experimental and calculated ECD) for all compounds and computational optimized *Z*-matrixes, thermodynamic parameters, and frequencies for **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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