Improved Synthesis of Isogranulatimide, a G2 Checkpoint Inhibitor. Syntheses of Didemnimide C, Isodidemnimide A, Neodidemnimide A, 17-Methylgranulatimide, and Isogranulatimides A−C

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A concise, improved synthesis of isogranulatimide (**6**), a naturally occurring substance with G2 checkpoint inhibition activity, is described. Also reported are the syntheses of didemnimide C (**18**), isodidemnimide A (**24**), neodidemnimide A (**36**), 17-methylgranulatimide (**9**), and isogranulatimides A (**10**), B (**11**), and C (**12**). Compounds **9**−**12**, congeners of isogranulatimide (**6**), are now available for biological evaluation.

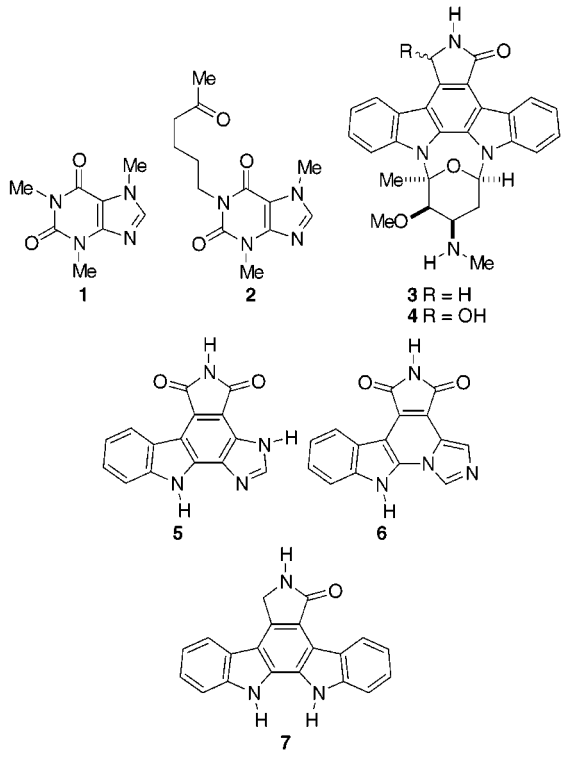
Introduction

Cells use a series of checkpoints to temporarily halt cell cycle progression in order to allow time for repair of damaged DNA.[1] The G1 checkpoint prevents damaged DNA from being replicated in S phase and the G2 checkpoint prevents damaged chromosomes from being segregated in mitosis. Many human cancers have genetic defects in their p53 tumor suppressor gene. These p53− cancers completely lack a G1 checkpoint and have partially defective G2 checkpoints.[2] It has been proposed that combination therapy utilizing a specific inhibitor of the G2 checkpoint and a DNA damaging agent would selectively target p53− cancer cells relative to normal p53+ cells.[3] This approach to increasing the therapeutic index of radiation or chemotherapy treatments seeks to take advantage of a well-defined genetic abnormality found in roughly half of all human cancers.

Two classes of G2 checkpoint inhibitors have been discovered serendipitously. One class includes the purine alkaloids caffeine (**1**) and pentoxifylline (**2**),[4] and the other includes the bisindolemaleimides staurosporine (**3**) and UCN-01 (**4**)[5] (Chart 1). In vitro experiments using a variety of paired cell lines differing only in their p53 status have shown that caffeine, pentoxifylline, and UCN01 induce greater sensitivity to DNA damage in p53− cells than in p53+ cells, validating the potential usefulness of this approach.[6] However, staurosporine and UCN01 are nonselective kinase inhibitors, and the purine alkaloids have multiple pharmacological activities. Consequently, none of the substances belonging to these classes of compounds are sufficiently selective G2 checkpoint inhibitors to test the principle of combination therapy in vivo. Therefore, it is important to find new selective G2 checkpoint inhibitors to further investigate this promising approach to cancer treatment.

Recently, we have used a novel high throughput assay to screen marine natural product extracts for metabolites

Chart 1



(1) (a) Hartwell, L.; Weinert, T.; Kadyk, L.; Garvick, B. *Cold Spring Harbor Symp. Quantum Biol.* **1994**, *59*, 259. (b) Kaufmann, W. K.; Paules, R. S. *FASEB J.* **1996**, *10*, 238.

(2) (a) Kastan, M. B.; Onyekwere, O.; Sidransky, D.; Vogelstein, B.; Craig, R. W. *Cancer Res.* **1991**, *51*, 6304. (b) Paules, R. S.; Levedakalou, E. N.; Wilson, S. J.; Innes, C. L.; Rhodes, N.; Tisty, T. D.; Galloway, D. A.; Donehower, L. A.; Tainsky, M. A.; Kaufmann, W. K. *Cancer Res.* **1995**, *55*, 1763.

(3) (a) Murray, A. W. *Nature* **1992**, *359*, 599. (b) Weinert, T.; Lydall, D. *Cancer Biol.* **1993**, *4*, 129. (c) Nurse, P. *Cell* **1997**, *91*, 865.

(4) (a) Busse, P. M.; Bose, S. K.; Jones, R. W.; Tolmach, L. J. *Radiat. Res.* **1978**, *76*, 292. (b) Schlegel, R.; Pardee, A. B. *Science* **1986**, *232*, 1264. (c) Downes, C. S.; Musk, S. R. R.; Watson, J. V.; Johnson, R. T. *J. Cell Biol.* **1990**, *110*, 1855. (d) Andreassen, P. R.; Margolis, R.L. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2272.

(5) (a) Tam, S. W.; Schlegel, R. *Cell Growth Differ.* **1992**, *3*, 811. (b) Wang, Q.; Fan, S.; Eastman, A.; Worland, P. J.; Sausville, E. A.; O'Connor, P. M. *J. Nat. Can. Inst.* **1996**, *88*, 956.

(6) (a) Fan, S.; Smith, M. L.; Rivet, D. J.; Duba, D.; Zhan, Q.; Kohn, K. W.; Firnace, J. A.; O'Connor, P. M. *Cancer Res.* **1995**, *55*, 1649. (b) Russell, K. J.; Weins, L. W.; Demers, G. W.; Galloway, D. A.; Plon, S. E. Groudine, M. *Cancer Res.* **1995**, *55*, 1639. (c) Powell, S. N.; DeFrank, J. S.; Connell, P.; Eogan, M.; Preffer, F.; Dombkowski, D.; Tang, W.; Friend, S. *Cancer Res.* **1995**, *55*, 1643. (d) Bunch, R. T.; Eastman, A. *Clin. Cancer Res.* **1996**, *2*, 791.

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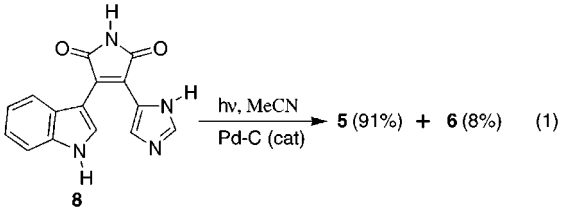
that inhibit the G2 checkpoint.[7] Extracts of the Brazilian ascidian *Didemnum granulatum* showed strong activity in the assay. Bioassay-guided fractionation of the extracts resulted in the isolation and structure elucidation of granulatimide (**5**) and isogranulatimide (**6**) (Chart 1), the first G2 checkpoint inhibitors discovered by rational screening.[7−9] The indole/maleimide/imidazole-containing aromatic heterocyclic skeletons of both **5** and **6** are without precedent among natural products, although they are related to the bisindolemaleimide-derived aglycon **7** of staurosporine. Interestingly, even though staurosporine (**3**) is a potent G2 checkpoint inhibitor, the staurosporine aglycon **7** is completely inactive. This is in marked contrast to granulatimide (**5**) and isogranulatimide (**6**), suggesting that the imidazole moiety in **5** and **6** plays a central role in their G2 checkpoint activity.

As part of the investigations leading to the structural elucidation of **5** and **6**, a short, efficient synthesis of granulatimide (**5**) was developed.[8,10] The final step of this synthesis also generated isogranulatimide (**6**), albeit in very low yield. Thus, irradiation (MeCN, medium-pressure mercury vapor lamp) of didemnimide A (**8**) in the presence of 10% palladium-on-carbon afforded **5** and **6** in isolated yields of 91 and 8%, respectively (eq 1).[8] The



primary objectives of the current study were (a) the development of an alternative protocol that would result in an efficient conversion of **8** into **6**, (b) the exploration of the generality of condensation−cyclization routes developed in the previous[8] and the present work (objective a, above), and (c) the preparation of structural analogues of **5** and **6**, including 17-methylgranulatimide (**9**) and substances **10**−**12** (Chart 2), in which the imidazole unit is incorporated into the final products in structural ways different from those present in **5** and **6**. For the sake of convenience, we have dubbed substances **10**−**12** isogranulatimide A, isogranulatimide B, and isogranulatimide C, respectively. Successful completion of these goals would set the stage for investigations into the effect of structure on the biological activity of this potentially important family of compounds.

Chart 2

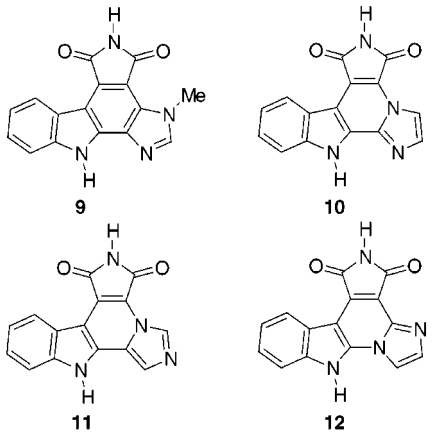
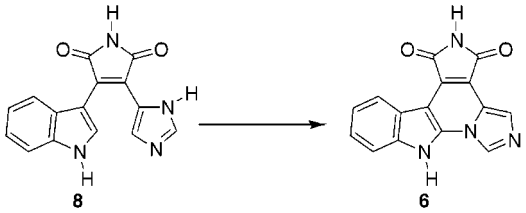


Table 1. Conversion of Didemnimide A (8) into Isogranulatimide (6)



entry	solvent	*T* (°C)	time (h)	conditions or additive	recovered **8** (%)	yield of **6** (%)
1	DMSO	140	2	open to atmosphere	22	51
2	$C_6H_5NO_2$	200	3	Pd/C[a]	22	46
3	$C_6H_5NO_2$	200	7	$Pd/CaCO_3$[b]	0	46
4	$C_6H_5NO_2$	200	8	Pd black[c]	28	62
5	$C_6H_5NO_2$	200	20	Pd black[c]	0	75

[a] Approximately 0.2 g of 10% Pd/C per mmol of **8**. [b] Approximately 0.4 g of 5% $Pd/CaCO_3$ per mmol of **8**. [c] Approximately 0.1 g of Pd black per mmol of **8**.

Results and Discussion

Synthesis of Isogranulatimide (6). Repetition of the reaction shown in eq 1[8] (vide supra) disclosed that the amount of isogranulatimide (**6**) produced from this process was variable. Indeed, the yields of **6** were typically even lower than that indicated in eq 1, and in some experiments, this substance was not present in the crude reaction mixture at all. These observations led to the conclusion that, in contrast to our previous suggestion,[8] the conversion of **8** into **6** is a thermally induced process. To gain further insight into this transformation, a number of high-temperature ^{1}H NMR spectroscopic experiments were performed, using $(CD_3)_2SO$ as the solvent. For example, the spectrum obtained when a solution of **8** was heated to 120 °C showed, in addition to signals due to the starting material, resonances that revealed the presence of isogranulatimide (**6**). The latter material became essentially the exclusive compound in solution when the mixture was heated to 160 °C.

These observations led to the preparative-scale experiments summarized in Table 1. It was found that heating of a solution of didemnimide A (**8**) in DMSO (140 °C, open to the atmosphere) provided isogranulatimide (**6**) in a maximum yield of about 50%. In addition, starting material was recovered in amounts varying from 0 to 20%, depending on reaction times (see, for example, Table 1, entry 1). Presumably, the transformation of **8** into **6**

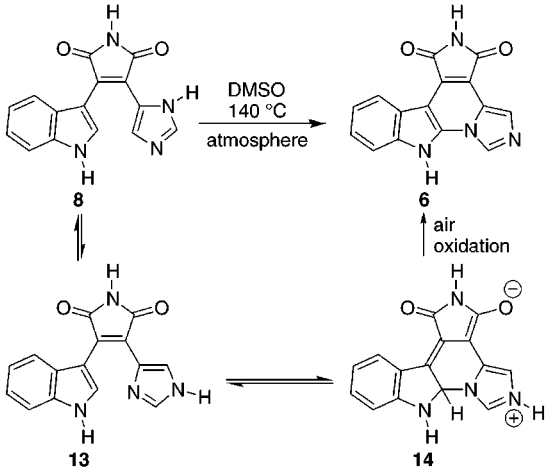
(7) Roberge, M.; Berlinck, R. G. S.; Xu, L.; Anderson, H.; Lim, L.; Curman, D.; Stringer, C. M.; Friend, S. H.; Davies, P.; Vincent, I.; Haggarty, S. J.; Kelly, M. T.; Britton, R.; Piers, E.; Andersen, R. J. *Cancer Res.* **1998**, *58*, 5701.

(8) Berlinck, R. G. S.; Britton, R.; Piers, E.; Lim, L.; Roberge, M.; da Rocha, R. M.; Andersen, R. J. *J. Org. Chem.* **1998**, *63*, 9850.

(9) After our paper[8] regarding the isolation and total synthesis of isogranulatimide was published, a report disclosing the isolation of a natural product identical with isogranulatimide was reported by others: Vervoort, H. C.; Fenical, W.; Keifer, P. A. *J. Nat. Prod.* **1999**, *62*, 389.

(10) A number of reports relating to the synthesis of substituted maleimides via methodology similar to that reported herein and in ref 8 have appeared in the literature since our original paper was submitted: (a) Faul, M. M.; Winneroski, L. L.; Krumrich, C. A. *J. Org. Chem.* **1998**, *63*, 6053. (b) Eils, S.; Winterfeld, E. *Synthesis* **1998**, 275. (c) Hughes, T. V.; Cava, M. P. *Tetrahedron Lett.* **1998**, *39*, 9629. (d) Faul, M. M.; Winneroski, L. L.; Krumrich, C. A. *Tetrahedron Lett.* **1999**, *40*, 1109. (e) Faul, M. M.; Winneroski, L. L.; Krumrich, C. A. *J. Org. Chem.* **1999**, *64*, 2465.

Scheme 1

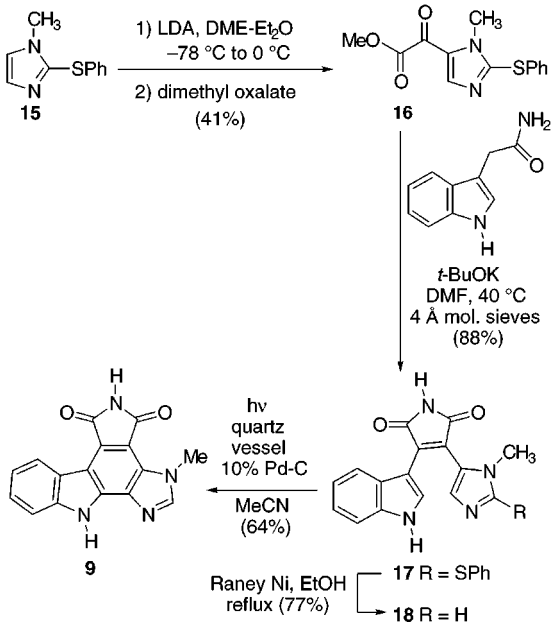


proceeds via (reversible) intramolecular 1,6-addition of **13** (a tautomer of **8**) to generate the intermediate **14**, as shown in Scheme 1. Air oxidation of the latter species (or of a structurally related intermediate derived from **14**) would regenerate the indole system and thus provide isogranulatimide (**6**) (Scheme 1). Unfortunately, further experimentation employing DMSO as solvent failed to identify conditions that would provide yields of **6** in excess of about 50%. The reactions consistently produced, in addition to **6**, insoluble (decomposition) material that was not investigated further.

Nitrobenzene is a well-known[11] hydrogen acceptor in hydrogen transfer reactions. It could be shown that isogranulatimide (**6**) is quite stable for extended periods of time in refluxing nitrobenzene and, therefore, a number of experiments involving this solvent were performed. When a solution of **8** in hot (200 °C) nitrobenzene containing 10% Pd/C was stirred for 3 h, **6** was produced in 46% yield, accompanied by the starting material **8** (22%) (Table 1, entry 2). The relatively low material balance from this reaction can probably be attributed to adsorption of **8** and **6** on the carbon support. Replacement of the Pd/C by Pd/$CaCO_3$ and increasing the reaction time to 7 h resulted in complete consumption of the starting material but produced the product **6** in moderate yield (entry 3). The rather "dirty" reaction mixture indicated that partial decomposition of the organic substances present had occurred. Additional experimentation showed that a satisfactory result could be obtained by using Pd black as the hydrogen transfer agent (entries 4 and 5). Indeed, although extended reaction times were required, the conditions summarized in entry 5 consistently transformed **8** into **6** in very good yields (~75%). Thus, an experimentally straightforward and efficient synthesis of isogranulatimide (**6**) had been developed, allowing the production of quantities sufficient for biological activity evaluations.

Syntheses of Didemnimide C (18) and 17-Methylgranulatimide (9). As mentioned above, one of the objectives of the current study was to carry out the syntheses of a number of substances (Chart 1) structurally related to granulatimide (**5**) and isogranulatimide (**6**). On the basis of our earlier work,[8] it was expected

Scheme 2



that one of the proposed targets, 17-methylgranulatimide (**9**), should be readily produced by photocyclization-oxidation[8] of didemnimide C (**18**).[12] Although a total synthesis of **18** had been achieved by Steglich and coworkers,[13] it seemed likely that a shorter synthesis, involving use of the methodology previously devised,[8] could be developed. The results derived from pursuing this approach are summarized in Scheme 2.

Sequential treatment of 1-methyl-2-phenylthioimidazole (**15**)[14] with LDA and dimethyl oxalate provided the required oxalate derivative **16** as the major product, accompanied by a number of minor, unidentified byproducts. Pure **16**, readily obtained by flash chromatography of the crude product mixture on silica gel, was produced in 41% yield. Condensation of **16** with indole-3-acetamide in the presence of *t*-BuOK and 4 Å molecular sieves[8] gave an excellent yield of the substituted maleimide **17**, which, upon desulfurization with Raney nickel in EtOH, provided didemnimide C **18** (3 steps from **15**, 28% overall yield). The ^{13}C NMR spectral data derived from this material agreed with those reported by Steglich and coworkers[13] for their synthetic material.

Subjection of didemnimide C (**18**) to photocyclization in the presence of Pd−C[8] provided 17-methylgranulatimide (**9**) in 64% yield. The relatively low efficiency of the **18** → **9** transformation is likely due to very low solubility of the latter compound in organic solvents. The presence of **9** in natural extracts of the ascidian *Didemnum granulatum* is presently being reinvestigated, since it is clear that it would be very difficult to extract this material from the tissue of the ascidian with commonly used solvents such as methanol or ethyl acetate.

As expected, the ^1H NMR spectrum of **9** is very similar to that of granulatimide (**5**), except that the resonance

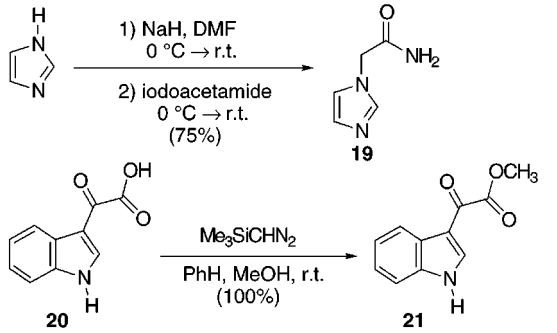
(11) (a) Brieger, G.; Nestrick, T. J. *Chem. Rev.* **1974**, *74*, 567. (b) Rawal, V. H.; Jones, R. J.; Cava, M. P. *Tetrahedron Lett.* **1985**, *26*, 2423.

(12) Vervoort, H. C.; Richards-Gross, S. E.; Fenical, W.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1997**, *62*, 1486.

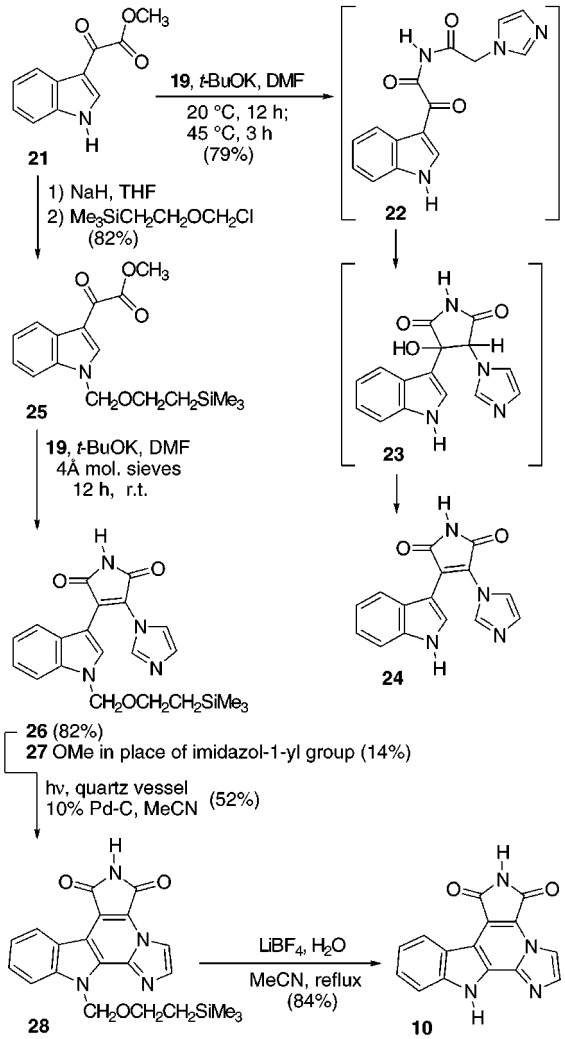
(13) Terpin, A.; Winklhofer, C.; Schumann, S.; Steglich, W. *Tetrahedron* **1998**, *54*, 1745.

(14) Ohta, S.; Yamamoto, T.; Kawasaki, I.; Yamashita, M.; Katsuma, H.; Nasako, R.; Kobayashi, K.; Ogawa, K. *Chem. Pharm. Bull.* **1992**, *40*, 2681.

Scheme 3



Scheme 4



due to the imidazole *N*-Me function in **9** (δ 4.31) replaces the corresponding *N*-H signal (δ 13.57) of **5**. Interestingly, as in the spectrum of **5**, the resonance due to the indole C-4 proton in **9** appears at δ 8.94. Furthermore, the imidazole *N*-Me groups of **18** and **9** give rise to singlets at δ 3.17 and 4.31, respectively. These data clearly demonstrate the deshielding effects of the neighboring maleimide carbonyl groups on the indole C-4 proton and the imidazole *N*-Me group in the planar structure **9**.

Syntheses of Isodidemnimide A (24), Isogranulatimide A (10), and Isogranulatimide B (11). Substance **24** (Scheme 4) is isomeric with didemnimide A (**8**) and, consequently, we have named the former compound isodidemnimide A. On the basis of the earlier studies,[8] it seemed probable that **24** could be conveniently prepared by a base promoted condensation of the amide **19** with the methyl glyoxylate **21**. It also seemed likely that **24**, or suitable derivatives thereof, would serve as convenient synthetic precursors to isogranulatimides A (**10**) and B (**11**).[15]

The syntheses of **19** and **21** are summarized in Scheme 3. Conversion of imidazole into the required amide **19** was effected via a procedure modified from that reported by Sundberg et al.[16] Although esterification of 3-indoleglyoxylic acid (**20**) with methanol in the presence of an ion-exchange resin has been reported,[10a] we have found that the conversion of **20** into **21** can be accomplished conveniently and quantitatively by use of trimethylsilyldiazomethane[17] (Scheme 3).

Scheme 4 outlines the use of substances **19** and **21** for the syntheses of isodidemnimide A (**24**) and isogranulatimide A (**10**). Potassium *tert*-butoxide-mediated condensation of **21** and **19** in warm (~45 °C) DMF gave modest yields (~30%) of the expected product isodidemnimide A (**24**). Examination of the reaction mixture revealed that a significant side product was *N*,*N*-dimethyl-3-indoleglyoxylamide. Presumably, this material resulted from reaction of **21** with $KNMe_2$, which, in turn, had been produced by reaction of the solvent (DMF) with *t*-BuOK. This unsatisfactory result was ameliorated by carrying out the initial steps of the condensation process at room temperature. Under these conditions, the starting materials **19** and **21** were consumed, but the expected product **24** was not produced. It was thus assumed that the desired condensation reactions (**19** + **21** → **22** → **23**) had taken place but that the final (dehydration) step (**23** → **24**) of the overall transformation had not yet occurred. Indeed, when the reaction mixture was subsequently warmed to 45 °C, isodidemnimide A (**24**) was produced in very good yield (Scheme 4).

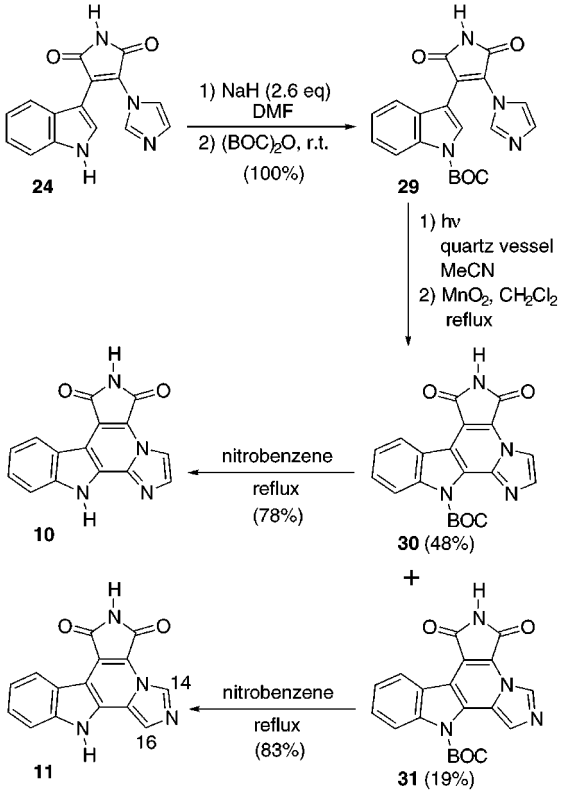
Attempts to convert **24** into isogranulatimides A (**10**) and/or B (**11**) via the previously employed photocyclization−oxidation protocol[8] were notably unsuccessful. Both the starting material **24** and the products **10** and **11** are sparingly soluble in acetonitrile, and various attempts to effect the photocyclization reaction consistently produced intractable material that was very difficult to cope with experimentally. Consequently, attention was directed toward the preparation and cyclization of suitable derivatives of **24**. To that end, treatment of **21** with NaH in THF, followed by reaction of the resultant anion with (2-trimethylsilylethoxy)methyl chloride (SEM-Cl), furnished the SEM derivative **25** (Scheme 4). Condensation of the latter material with the amide **19** in the presence of 4 Å molecular sieves[8] produced a high yield (82%) of the desired 1-(SEM)-isodideminimide A (**26**), accompanied by a minor amount (14%) of the methoxy derivative **27**. Presumably, **27** was produced by reaction (conjugate addition−β-elimination) of the maleimide **26** with methoxide ion, which is produced during the condensation of **25** with **19**. It should be noted that when the reaction was carried out in the absence of molecular sieves, **27** was the major product. It is likely that, to a large degree,

(15) For the synthesis of substituted versions of **10** and **11**, see ref 13.

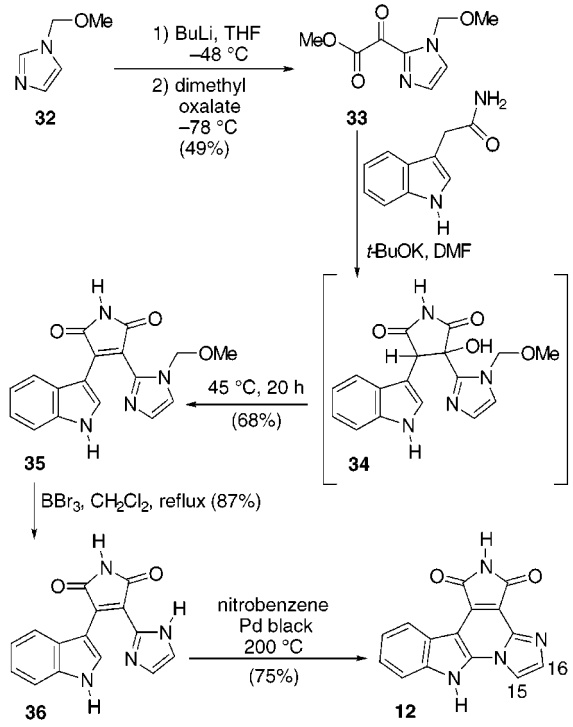
(16) Sundberg, R. J.; Mente, D. C.; Yilmaz, I.; Gupta, G. *J. Heterocycl. Chem.* **1977**, *14*, 1279.

(17) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475.

Scheme 5



Scheme 6



the sieves adsorb the methanol generated during the reaction and thus are effective in significantly reducing the amount of **27** produced.

The SEM derivative **26** is considerably more soluble in organic solvents than the parent isodidemnimide A (**24**). Irradiation of a solution of **26** in MeCN in the presence of 10% Pd−C[8] produced, after chromatographic purification of the crude product, 1-(SEM)-isogranulatimide A (**28**) in 52% yield, accompanied by a trace amount of the isomeric 1-(SEM)-isogranulatimide B. Although the reaction was not particularly clean (a number of minor unidentified byproducts were produced), the chromatographic acquisition of pure **28** was straightforward. The fact that the major mode of cyclization had involved C-2 of the imidazole ring was shown clearly by performing suitable ^{1}H NMR nuclear Overhauser enhancement difference (NOED) experiments on the product **28**. In the spectrum of **28**, the resonances of the imidazole protons, appear at δ 7.87 and 8.49. Irradiation at δ 7.87 caused enhancement of the signal at δ 8.49 and vice versa. Finally, reaction of **28** with $LiBF_4$ in MeCN containing a small amount of water[18] furnished isogranulatimide A (**10**) in excellent yield (Scheme 4).

Scheme 5 summarizes the use of 1-(BOC)-isodidemnimide A (**29**) as an intermediate for the syntheses of both isogranulatimides A (**10**) and B (**11**). Sequential treatment of isodidemnimide A (**24**) with sodium hydride (2.6 equiv) and di-*tert*-butyl dicarbonate resulted in a chemoselective reaction at the indole nitrogen and produced **29** in quantitative yield. After some preliminary experimentation, it was found that irradiation of a solution of **29** in MeCN with a 275 W light bulb for 1 h, followed by treatment of the resultant crude product with manganese dioxide in refluxing dichloromethane,[13] afforded a mixture of products. Flash chromatography of this material on silica gel provided 1-(BOC)-isogranulatimide A (**30**) and 1-(BOC)-isogranulatimide B (**31**) in yields of 48 and 19%, respectively.

Removal of the BOC function from **30** and **31** was conveniently achieved[19] by refluxing nitrobenzene solutions of each of these substances for 1 h. The corresponding products, isogranulatimides A (**10**) and B (**11**), were obtained in yields of 78 and 83%, respectively. The ^{1}H NMR spectrum of the former compound is identical with that of the same substance obtained as described above (Scheme 4). On the other hand, the spectrum of **11** clearly showed that this material is isomeric with **10**. In the ^{1}H NMR spectrum of **11**, the two imidazole protons give rise to singlets at δ 8.87 (C-14 proton) and 7.94 (C-16 proton). In key NOED experiments, irradiation at δ 7.94 increased the intensity of the indole *N*-H resonance (δ 12.99) and vice versa.

Syntheses of Neodidemnimide A (36) and Isogranulatimide C (12). The syntheses of substances **36** (which we have named neodidemnimide A) and **12** (isogranulatimide C) are summarized in Scheme 6. A review of the chemical literature[20] relating to the metalation of imidazoles shows that there exists a disparity in the efficiency of the acylation of C-2 lithio derivatives of *N*-substituted imidazoles. Although some success has been realized through the use of tertiary amides[20a] or of the sterically bulky pivaloyl chloride,[20b] the use of less hindered acid chlorides or anhydrides[20c] have generally provided low yields of the desired 2-acyl adducts. Consequently, it was not particularly surprising to find that reaction of 1-methoxymethylimidazole (**32**)[21] with BuLi

(18) Ireland, R. E.; Varney, M. D. *J. Org. Chem.* **1986**, *51*, 635.

(19) Rawal, V. H.; Cava, M. P. *Tetrahedron Lett.* **1985**, *26*, 6141.

(20) (a) Ohta, S.; Hayakawa, S.; Moriwaki, H.; Tsuboi, S.; Okamoto, M. *Heterocycles* **1985**, *23*, 1759. (b) Chadwick, D. J.; Ngochindo, R. I. *J. Chem. Soc., Perkin Trans. 1* **1984**, 481. (c) Lipshutz, B. H.; Vaccaro, W.; Huff, B. *Tetrahedron Lett.* **1986**, *27*, 4095. (d) Iddon, B.; Ngochindo, R. I. *Heterocycles* **1994**, *38*, 2487.

(21) Roe, A. M. *J. Chem. Soc.* **1963**, 2195.

in THF, followed by addition of dimethyl oxalate, provided a mixture of products. Nevertheless, column chromatography of the mixture allowed the ready isolation of the required oxalate **33** in 49% yield. The major byproduct was the diketone produced by reaction of **33** with a second equivalent of the anion derived from **32**. Interestingly, base-mediated condensation of **33** with indole-3-acetamide in DMF required extended reaction times at 45 °C to effect efficient formation of the neodidemnimide derivative **35**. For example, when a DMF solution of **33**, indole-3-acetamide, and *t*-BuOK was stirred at room temperature overnight and then was heated at 45 °C for about 4 h (see **21** + **19** → **24**, Scheme 4), little of the product **35** was formed. A spectroscopic examination of the components of the reaction mixture indicated that the major product at this stage was the hydroxy succinimide **34** and that, therefore, the required dehydration step (**34** → **35**) had not yet taken place. On the other hand, when the reaction mixture was stirred at 45 °C for 20 h, 14-methoxymethylneodidemnimide A (**35**) was obtained in 68% yield.

The conversion of **35** into neodidemnimide A (**36**) by use of BBr_3 in refluxing CH_2Cl_2[8] was clean and efficient. When a solution-suspension of **36** and Pd black in nitrobenzene was heated at 200 °C under an atmosphere of argon, isogranulatimide C (**12**) was produced in good yield.

The ^{1}H NMR spectroscopic data derived from **12** fully supported the structural assignment. The ^{1}H NMR spectrum of **12** displays signals for the two imidazole protons at δ 7.93 (C-16 hydrogen) and 8.40 (C-15 hydrogen). In key NOED experiments, irradiation at δ 7.93 increased the intensity of the 8.40 signal, while irradiation at δ 8.40 enhanced the signals at 7.93 and 13.36 (indole *N*-H),

Conclusion

It has been established[7,8] that granulatimide (**5**) and isogranulatimide (**6**) are effective G2 specific cell cycle checkpoint inhibitors. Consequently, these novel natural products could play an important future role in the treatment of cancer. Clearly, it was desirable to synthesize a number of compounds structurally related to **5** and **6**, so that a larger number of constitutionally similar substances could be subjected to testing for G2 checkpoint inhibition. The work described in this paper culminated in a much improved synthesis of isogranulatimide (**6**) and in the syntheses of a number of congeners of **5** and **6**, including 17-methylgranulatimide (**9**) and isogranulatimides A, B, and C (**10**, **11**, and **12**, respectively). The results derived from evaluations of the biological activity of these materials will be reported elsewhere.

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Supporting Information Available: Experimental procedures and spectral data for compounds **6**, **9**−**12**, **16**−**19**, **21**, **24**−**26**, **28**−**30**, **33**, **35**, and **36** and ^{1}H NMR spectra of compounds **9**−**12**, **16**, **17**, **24**−**26**, **28**−**30**, **33**, **35**, and **36**. This material is available free of charge via the Internet at http://pubs.acs.org.

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