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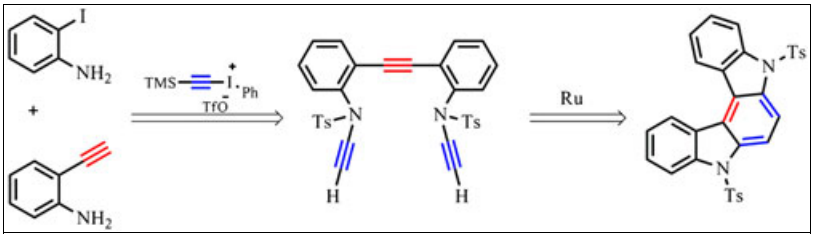

The dictyodendrins A–E were the first marine natural products that show inhibition of telomerase. A versatile and convergent route was described for the synthesis of derivatives of these pyrrolo[2,3-*c*]carbazole alkaloids as potential inhibitors of telomerase, by cyclotrimerization [2 + 2 + 2] of ruthenium-catalyzed diynamides diarylacetylenes.

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INTRODUCTION

Telomeres are highly repetitive uncoded DNA segments located at the extremes of the chromosomes of eukaryotic cells where they mainly function as chromosomal structural stabilizers [1–4]. In humans, telomeres present the following sequence: TTAGGG. It is known that successive cellular divisions incur in the loss of telomeric repetitions (50–70 nucleotides) that limits the useful life span of normal somatic cells to 50–70 cellular divisions; consequently, the telomeric moiety loss has been proposed as a cellular "mitotic clock" [2,5–7].

The telomere replication process relies on the telomerase, a complex ribonucleic protein that incorporates an RNA component (TERC) and a reverse transcriptase catalytic subunit (TERT) [8,9].

The correlation between telomerase and cancer was established when an overexpression of this enzyme was observed in most human tumors (85–90%), in contrast to healthy cells [10]. Preclinical studies have demonstrated that telomerase inhibition in selected cancerous human cells can halt cell development and induce apoptosis as a result of telomere-induced crisis [11].

Some molecules are known inhibitors of telomerase; however, there are only few. That is the reason why the study of natural and synthetic metabolites possessing telomerase inhibitory activity has potential. During the last decades, the metabolites of marine origin have not been studied in detail; therefore, they have become one of the most promising clues for the anticancer pharmaceutical development.

Fusetani *et al*. isolated and identified five new alkaloids from the marine sponge *Dictyodendrilla verongiformis* collected in southern Japan. These metabolites called dictyodendrins showed 100% inhibition of telomerase activity at a concentration of 50 μg/mL. Dictyodendrins were the first marine natural products to inhibit telomerase [12,13]. These tyramine-derived alkaloids contain a unique and characteristic pyrrolo[2,3-*c*]carbazole nucleus comprised of electron-rich aromatic rings and at least one peripheral sulfate group (Fig. 1). Fürstner and collaborators proposed total synthetic routes for three of the five isolated dictyodendrins [14,15].

Recent publications have revealed the foregoing study and interest in the synthesis of indole and pyrrolocarbazole structures present in natural products that exhibit diverse biological properties. For example, Fousteris has described the synthesis of pyrrolo[2,3-*a*]carbazoles by means of trimethylsilyl polyphosphate catalyzed Fisher indole cyclization of arylhydrazones [16,17]. Ayats described the synthesis of pyrrolo[2,3-*c*]carbazoles based on a Suzuki cross-coupling followed by a 6π-electrocyclization [18–24].

The increasing popularity of metal-catalyzed [2 + 2 + 2] cycloaddition reactions derives from the ability of this methodology to employ a vast array of starting substrates [25–27]. Saá and coworkers have investigated [2 + 2 + 2] cycloadditions of alkyne and nitriles catalyzed by cobalt and ruthenium metal complexes for the synthesis of pyridines [28–32], as well as ruthenium-catalyzed formation of 1,2-cyclohexadienes from 1,6-dienes, and alkenes [33–35].

RESULTS AND DISCUSSION

In search of new alternatives for cancer treatment, we decided to attempt to synthesize dictyodendrin analogs with 5,8-dihydroindolo[2,3-*c*]carbazole structures as potential

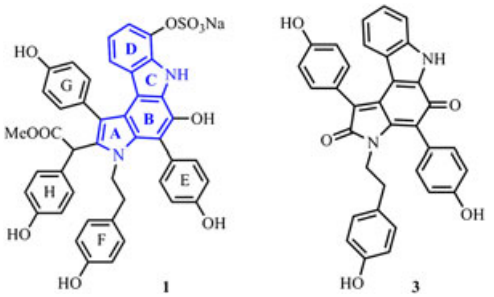


Figure 1. Dictyodendrin **A** (**1**) and dictyodendrin **C** (**3**) isolated from the marine sponge *Dictyodendrilla verongiformis* [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

telomerase inhibitors. These organic structural units might be accessible through metal-catalyzed [2 + 2 + 2] cycloadditions of diynamides diarylacetylenes.

The synthesis of the diynamide diarylacetylene first required the preparation of 2-ethynylaniline **5** by a two-step sequence involving the Sonogashira coupling of 2-iodoaniline **4** with trimethylsilylacetylene to obtain the silylated intermediate [36] and then subject the product to basic (K_2CO_3) methanolic desilylation resulting in **5** at 84% overall yield.

Finally, ditosyl dianilide **6** was obtained at 75% yield by means of Sonogashira coupling of **5** and **4** followed by *N*-tosylation of the resulting dianiline (Scheme 1) [37].

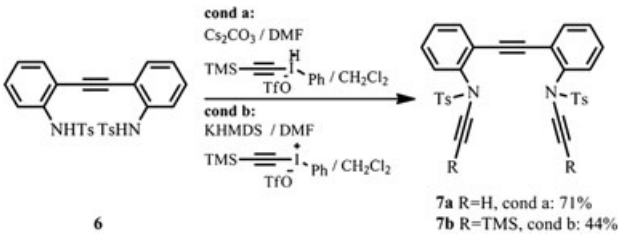
The employed (trimethylsilyl)ethynyliodonium salt for *N*-alkylation of **6** was prepared by a simple and high-yield procedure (85%) previously reported [38].

N-Ethynylation of **6** resulted in diynamide diarylacetylene **7a** when, cesium carbonate was used as base (Scheme 2, condition a), while diynamide diarylacetylene silylated **7b** and ynamide diarylacetylene silylated **8** (Fig. 2) were obtained when potassium bis(trimethylsilyl)amide (KHMDS) was used as base (Scheme 2, condition b) [39].

The ^{1}H-NMR spectrum of **7a** showed a singlet at 2.89 ppm that integrated for 2H and a signal at 2.22 ppm that integrated for 6H corresponding to the two alkene terminal hydrogen atoms and the two methyl hydrogen atoms of the tosyl groups, respectively. ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) resulted in a signal at 76.10 ppm for the quaternary carbon of the ynamide and the signal at 59.38 ppm related to the terminal CH of the ynamide.

In the ^{1}H-NMR spectrum of **7b**, one singlet at 0.00 ppm was observed [which corresponded to tetramethylsilane (TMS)], and a single high-field signal at 2.12 ppm that integrated for 6H corresponded to tosylmethyl hydrogen. In ^{13}C-NMR and

Scheme 2. Diynamide diarylacetylene synthesis pathway.



DEPT, the 0.18-ppm signal corresponded to TMS; the 73.42-ppm signal corresponded to the alkene carbon bonded to the nitrogen atom, and the 94.85-ppm signal related to the alkene carbon bonded to TMS.

In order to synthesize various dictyodendrin analogs, *N*,*N*′-(ethyne-1,2-diylbis(2,1-diphenylene))dipropiolamide **10** was prepared by *N*-acylation of dianiline **9** with propiolic acid and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) base; the dipropiolamide yield was 80% (Scheme 3).

A singlet at 2.99 ppm was observed in the ^{1}H-NMR spectrum, which integrated for 2H and corresponded to the terminal alkyne protons. A wider singlet at 8.21 ppm integrated for 2H and corresponded to the two N–H units.

In the ^{13}C-NMR and DEPT spectrum of compound **10**, the amide carbonyl signal was observed at 156.90 ppm and two quaternary carbon shifts at 111.84 and 91.42 ppm; the former corresponded to the alkyne carbon bonded to the carbonyl and the later to the alkyne substituted at both sides by phenyls. The signal at 74.96 ppm corresponded to the terminal alkyne carbon.

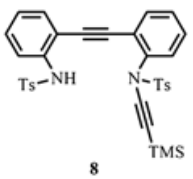
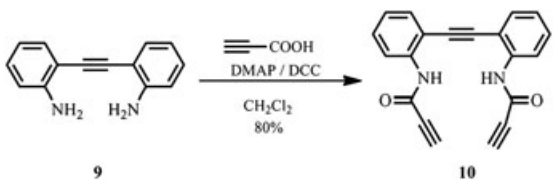
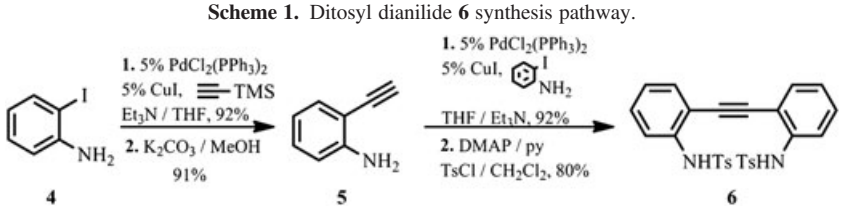


Figure 2. Ynamide diarylacetylene silylated.

Scheme 3. Dipropiolamide synthesis pathway.



Scheme 1. Ditosyl dianilide **6** synthesis pathway.



The treatment of diynamide diarylacetylene **7a** with the ruthenium-catalyzed $[Cp^*Ru(CH_3CN)_3]PF_6$ (10%) and Et_4NCl (10%) at room temperature resulted in the intramolecular [2 + 2 + 2] cycloaddition pentacyclic product **11a** with a yield of 65% (Scheme 4) [34].

A characteristic signal of the **11a** pentacycle was observed, a singlet signal in the ^{1}H-NMR at 2.23 ppm that integrated for 6H and that corresponded to the tosylmethyl groups. The singlet at 8.61 ppm that integrated for 2H corresponded to the H5 and H6 protons of 5,8-dihydroindolo[2,3-*c*]carbazole.

The ^{13}C-NMR and DEPT spectrum analysis indicated various signals in the aromatic region and a signal at 21.63 ppm that corresponded to the tosylmethyl groups, thus giving proof of the disappearance of alkyne signals of the starting compound **7a**.

Unfortunately, the cyclization attempts of **7b** and **10** employing the before mentioned ruthenium-catalyzed compound under the same conditions were unsuccessful as shown in Table 1. In the case of compound **7b**, only decomposition products were obtained. In addition, compound **10** remained unchanged.

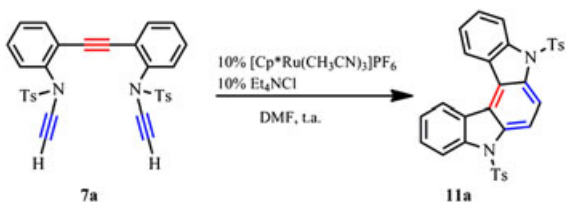
The presence of the bulky trimethylsilane group in **7b** disfavored the intramolecular [2 + 2 + 2] cycloaddition, and therefore, only decomposition products were obtained. In the case of compound **9**, the extra carbon bridge effectively produced a larger spatial separation between pendant alkynes, thus hindering the cycloaddition.

The proposed mechanism for ruthenium-catalyzed [2 + 2 + 2] cycloadditions is presented in Scheme 5 for the diynamide diarylacetylene compound **7a** [40,41].

The admixture of Et_4NCl with the cationic compound $[Cp^*Ru(CH_3CN)_3]PF_6$ gave rise to the true catalytic species $[RuL_2ClCp^*]$. The oxidative coupling of diene **7a** with the neutral ruthenium species produced ruthenacyclo heptabiscarbene **I**, which after coordination and insertion with the second alkyne unit, generated ruthenacyclo heptatriene **II**. Reductive elimination in **II** produced the pentacyclic product **11a**, thereby the regeneration of the catalytic species.

In conclusion, an efficient protocol for the synthesis of pyrrolo[2,3-*c*]carbazoles was developed by ruthenium-catalyzed [2 + 2 + 2] cyclotrimerization starting from diynamide diarylacetylenes. This chemical transformation is characterized by its mild reaction conditions and relatively good yields.

Scheme 4. Cycloaddition [2 + 2 + 2]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



7a **11a**

MATERIAL AND METHODS

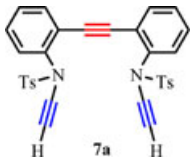
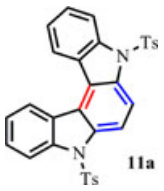
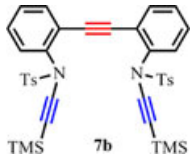
All reactions were carried out under argon atmosphere with magnetic stirring. The solvents were purified and dried using standard procedures. All reagents were purchased and used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were determined on Bruker DPX-250-MHz, AMX-300-MHz, Varian Mercury 300-MHz spectrometer using $CDCl_3$ as solvent with TMS as internal standard. Chemical shifts are expressed as δ ppm and J as Hz units. Mass spectra were measured by ionizing the sample at 70 eV. Column chromatography was made on silica gel 230–240 mesh (flash). Melting points were measured with a Büchi apparatus and are uncorrected.

Diynamide diarylacetylene synthesis 7a. To a stirred solution of ditosyl dianilide **6** (0.194 mmol) in dimethylformamide (DMF 4 mL), $CsCO_3$ (2.60 equiv)was added at room temperature (rt). After 30 min, a solution of the iodonium salt (2.60 equiv) in CH_2Cl_2 (10 mL) was added dropwise and was stirred at rt overnight. The mixture was diluted with diethyl ether and washed with brine and water. The aqueous phase was extracted with ether (3 × 10 mL) dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography using a gradient mixture of Hex : AcOEt as eluent. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.0 Hz, 5H), 7.32 (m, 4H), 7.30 (m, 2H), 7.17 (d, J = 8.0 Hz, 5H), 2.89 (s, 2H), 2.22 (s, 6H). ^{13}C-NMR and DEPT (101 MHz, $CDCl_3$) δ 145.23 (2 × C), 138.35 (2 × C), 134.49 (2 × C), 133.98 (2 × CH), 129.82 (4 × CH), 129.45 (4 × CH), 128.98 (2 × CH), 128.55 (4 × CH), 122.67 (2 × C), 91.09 (2 × C), 76.10 (2 × C), 59.38 (2 × CH), 21.67 (2 × CH_3). Mass spectroscopy [MS; electrospray ionization (ESI)] *m/z* (%): 565.13 (58), 409.10 (M^+ + 1, 100), 338.35 (10). Electromagnetic acoustic resonance (EMAR; ESI, M^+ + 1; $C_{32}H_{24}N_2O_4S_2$) calcd: 565.1250; found: 565.1257. Mp (°C): 156.3–156.9.

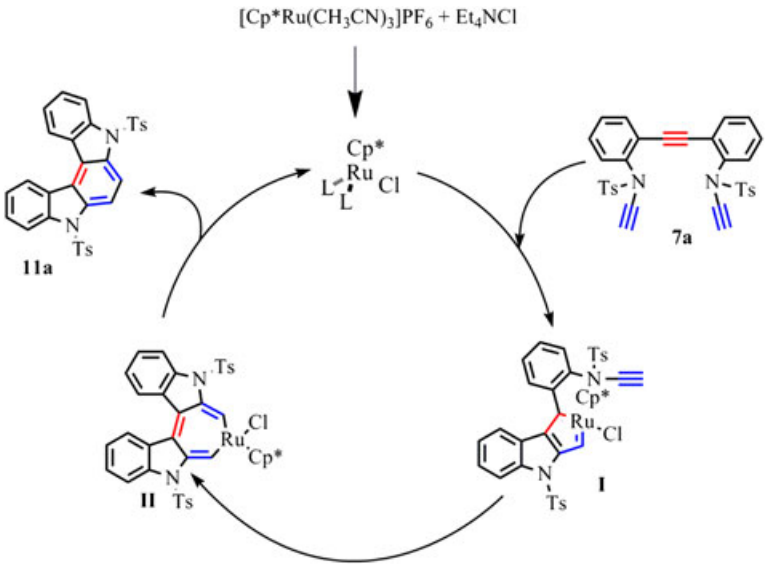
Diynamide diarylacetylene silylated 7b synthesis. To stirred a solution of ditosyl dianilide **6** (0.194 mmol) in toluene (0.093 *M*) at 0°C, KHMDS (0.47 mmol, 2.40 equiv) was added dropwise. After 30 min, a solution of the iodonium salt (0.54 mmol, 2.8 equiv) in CH_2Cl_2 (1.7 mL, 0.33 *M*) and the mixture were stirred at rt overnight. The solvent was evaporated, and the product was dissolved with diethyl ether and washed with brine. The aqueous phase was extracted with ether (3 × 10 mL) dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography eluting with Hex : AcOEt 8:2. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 7.63 (d, J = 8.2 Hz, 4H), 7.24 (m, 4H), 7.16 (m, 2H), 7.07 (t, J = 8.2 Hz, 6H), 2.12 (s, 6H), 0.00 (s, 18H). ^{13}C-NMR/DEPT (63 MHz, $CDCl_3$) δ 145.12 (2 × C), 138.60 (2 × C), 134.49 (2 × C), 134.01 (2 × CH), 129.58 (4 × CH), 129.51 (2 × CH), 129.30 (2 × CH), 128.78 (4 × CH), 128.51 (2 × CH), 122.36 (2 × C), 94.58 (2 × C), 91.28 (2 × C), 73.42 (2 × C), 21.65 (2 × CH_3), 0.18 (6 × CH_3). MS (ESI) *m/z* (%): 731.2 (M^+ + 1,

Table 1

Cycloaddition [2 + 2 + 2] according to Scheme 4.

Substrate	Catalyst	Product
	10% $[Cp^*Ru(CH_3CN)_3]PF_6$ 10% Et_4NCl DMF, rt	
	10% $[Cp^*Ru(CH_3CN)_3]PF_6$ 10% Et_4NCl DMF, rt	Decomposition products
	10% $[Cp^*Ru(CH_3CN)_3]PF_6$ 10% Et_4NCl DMF, rt	

Scheme 5. The cycloaddition [2 + 2 + 2] mechanism. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



83), 709.2 ($M^+ + 1$, 23), 481.2 (87), 415.2 (100). EMAR (ESI, $M^+ + 1$; $C_{38}H_{40}N_2O_4S_2Si_2$) calcd: 709.2041; found: 709.2011. Mp (°C): 188.0–188.5.

Dipropiolamide synthesis 10. A solution of dianiline (0.25 mmol), propiolic acid (0.60 mmol, 2.4 equiv), and DMAP (0.005 mmol, 0.02 equiv) in CH_2Cl_2 (2.2 mL, 0.117 *M*) was stirred at rt for 15 min, and 0.60 mmol (2.4 equiv) of N,N'-Dicyclohexylcarbodiimide was then added and stirred at rt overnight. The mixture was washed with HCl 5% and brine. The organic layer was dried over

Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography eluting with Hex : AcOEt 7:3. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.33 (d, $J = 8.3$ Hz, 2H), 8.21 (s, 2H), 7.55 (dd, $J = 7.7$ and 1.6 Hz, 2H), 7.43 (td, $J = 7.7$ and 1.6 Hz, 2H), 7.19 (t, $J = 7.7$ Hz, 2H), 2.99 (s, 2H). ^{13}C-NMR and DEPT (75 MHz, $CDCl_3$) δ 156.90 (2×C), 149.66 (2×C), 138.01 (2×C), 132.14 (2×CH), 130.74 (2×CH), 124.88 (2×CH), 120.80 (2×CH), 111.84 (2×C), 91.42 (2×C), 74.96 (2×C). MS (ESI) *m/z* (%): 233.1 (96), 255.2 (100), 285.0 (44), 311.2 (25).

Cycloaddition [2 + 2 + 2]. A mixture of ruthenium-catalyzed (0.0112 mmol, 0.1 equiv) compounds, $Et_4N^+Cl^-$ (0.0112 mmol, 0.1 equiv), and DMF (1 mL, 0.112 *M*) was stirred at rt for 45 min, and 0.063 g (0.1117 mmol) of diynamide was then added and stirred at rt for 4 h. The mixture was diluted with diethyl ether, filtered through silica, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel eluting with Hex : EtOAc (7:3) to give **11a** was performed. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 8.61 (s, 2H), 8.53 (m, 3H), 7.67 (d, $J = 8.4$ Hz, 4H), 7.53 (m, 5H), 7.06 (d, $J = 8.4$ Hz, 4H), 2.23 (s, 3H). ^{13}C-NMR and DEPT (63 MHz, $CDCl_3$) δ 145.17 (2×C), 139.06 (2×C), 136.10 (2×C), 134.73 (2×C), 129.78 (4×CH), 127.51 (2×CH), 126.60 (4×CH), 126.13 (2×C), 123.99 (2×CH), 123.21 (2×CH), 121.01 (2×C), 115.75 (2×CH), 114.50 (2×CH), 21.63 (2×CH_3). MS (ESI) *m/z* (%): 565.57 (2), 428.11 (22), 427.11 (100). EMAR (ESI, $M^+ + 1$; $C_{32}H_{24}N_2O_4S_2$) calcd: 565.1250; found: 565.5657. Mp (°C): 269.6–270.1.

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