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Brief Total Synthesis of the Cell Cycle Inhibitor Tryprostatin B and Related Preparation of Its Alanine Analogue

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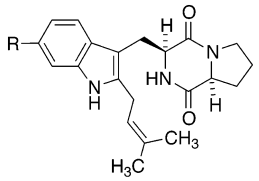
Tryprostatin B was synthesized in 32% overall yield from the readily available dipeptide anhydride *cyclo*-(L-Trp-L-Pro). Its tandem C-3 prenylation/cyclization gave the corresponding pentacyclic pyrroloindole systems bearing a prenyl group at the indole C-3 position. These compounds were then submitted to acid-catalyzed opening of the newly formed ring, with concomitant migration of the prenyl group to the indole C-2 position. The alanine analogue of tryprostatin B was also prepared using a similar sequence. The successful implementation of this strategy strengthens the case for a biosynthetic route for the tryprostatins along similar lines.

Introduction

The cell cycle is an attractive target for the development of chemotherapeutic agents, with much current interest being focused on the search for antimitotic agents.[1] Since cancer can be considered to arise from uncontrolled cell proliferation with loss of regulation of the cell cycle, new inhibitors of this cycle are good candidates for cancer chemotherapy[2] and can also be useful probes for investigating the control of the cell cycle.[3]

Tryprostatins A and B (**1** and **2**, Figure 1) are two natural products isolated from a marine strain (BM939) of *Aspergillus fumigatus*.[4] They can be considered as members of a small family of indole-isoprene alkaloids that, among others, includes compounds such as echinulin, from *Aspergillus echinulatus*,[5] the neoequinulines, from *Aspergillus amstelodami*,[6] and deoxybrevianamide E[7] and related compounds,[8] from *Aspergillus ustus*. The tryprostatins[9] and also some related compounds such as the diketopiperazine alkaloid phenylahistin,[10] the spirotryprostatins,[11] and the cyclotryprostatins[12] are inhibitors of the mammalian cell cycle. The tryprostatins prevent cell cycle progression at the G_2/M phase through a unique mechanism, consisting of inhibition of the interaction between one of the microtubule-associated proteins (MAP-2) and the C-terminal end of tubuline.[13] Recently, a diastereomer of tryprostatin B has shown a more potent cytotoxic activity than etoposide against three human cancer cell lines.[14a]



R = OCH_3 Tryprostatin A (**1**)
R = H Tryprostatin B (**2**)

FIGURE 1. Structures of Tryprostatins A and B.

The considerations outlined above prompted us to study a synthetic route to the tryprostatins, in particular tryprostatin B. All total[15−17] and formal[18] syntheses of the tryprostatins published to date rely on the prepara-

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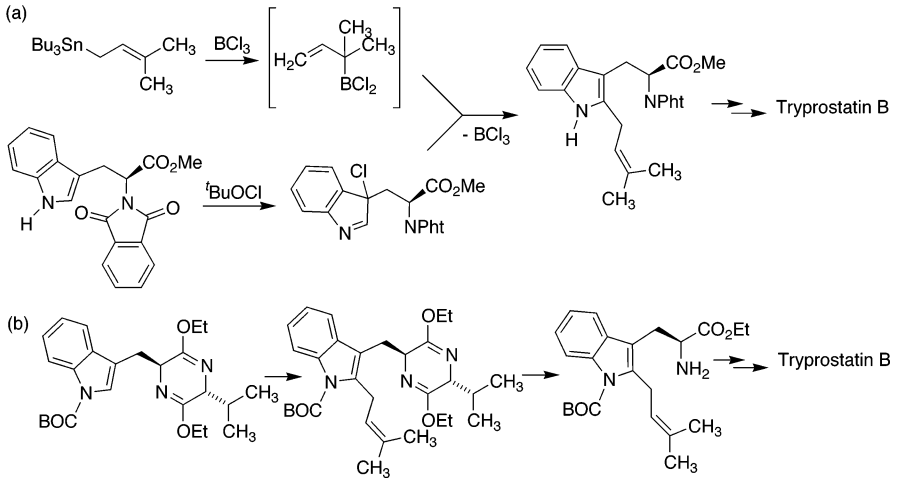
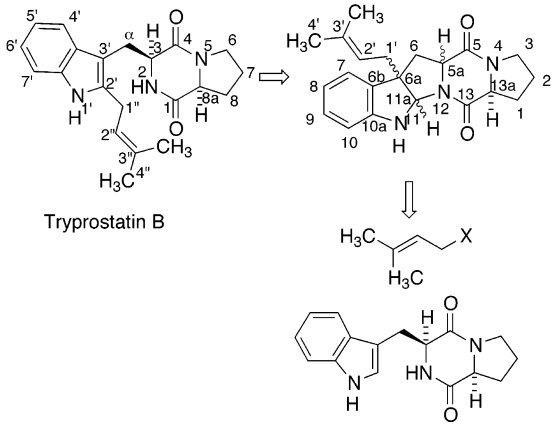
SCHEME 1. Previous Total Syntheses of Tryprostatin B



tion of a suitable 2-prenylindole intermediate. Regarding tryprostatin B itself, two total syntheses have been published. The one due to Danishefsky[15] is based on the preparation of a 2-prenyltryptophan derivative by attack of a nucleophilic species generated from tributylprenylstannane and boron trichloride onto an unstable 3-chloroindolenine, obtained from a suitably protected tryptophan derivative (Scheme 1a). The synthesis developed by Cook[16c] is also based on the preparation of a 2-prenyltryptophan derivative, in this case by directed ortholithiation of a *N*-BOC-indolymethyl derivative of the Schöllkopf chiral auxiliary, followed by alkylation and hydrolysis of the pyrazine moiety (Scheme 1b). This route has been subsequently adapted to work on a solid phase by attaching the prenylindole derivative to a resin through a silyl linker.[19] Also relevant are the alternative syntheses of tryprostatin B developed by Cook, which are also based on the multistep preparation of a suitable 2-prenyltryptophan, either by halogen−lithium exchange of a 2-bromo-3-methylindole derivative[16a,b] followed by alkylation and incorporation of the α-amino acid moiety using Schöllkopf chemistry or by a palladium-mediated heteroannulation reaction.[16d]

We describe here an alternative strategy for the synthesis of tryprostatin B based on the preparation of a fused pentacyclic compound from *cyclo*-(L-Trp-L-Pro), followed by rearrangement of the prenyl chain[20] with concomitant ring opening and rearomatization of the indole system (Scheme 2). By simply replacing L-Pro by other amino acids as the starting material, this route can be made amenable to the preparation of tryprostatin analogues.

SCHEME 2. Retrosynthetic Analysis of Tryprostatin B



To prepare the required prenylated pentacyclic intermediate from *cyclo*-(L-Trp-L-Pro), we initially considered the possibility of using the procedure developed by Crich for introducing a prenyl group at the 3a position of a hexahydropyrrolo[2,3-*b*]indole system during his synthesis of *ent*-debromoflustramine B,[21] which relies on the regioselective bromination of a benzylic position with preference to a captodative one. However, we have recently shown that cyclic tautomers of *cyclo*-(L-Trp-L-Ala) derivatives behave differently than hexahydropyrrolo[2,3-*b*]indoles toward bromination, which does not take place at the benzylic position as required.[22] We therefore turned our attention to the possibility of carrying out a tandem prenylation−cyclization of *cyclo*-(L-Trp-L-Pro). Tandem C-3 *protonation*/cyclizations of tryptophan and tryptamine derivatives[23] and 2,5-pipera-

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(20) For precedent of prenyl migration from C-3 to C-2 triggered by ring opening in related systems, see: (a) Nakagawa, N. M.; Matsuki, K.; Hasumi, K.; Taniguchi, M.; Hino, T. *Heterocycles* **1982**, *19*, 156 (symposium abstract). (b) Hino, T.; Hasumi, K.; Yamaguchi, H.; Taniguchi, M.; Nakagawa, M. *Chem. Pharm. Bull.* **1985**, *33*, 5202−5206. (c) Plate, R.; Ottenheijm, H. C. J. *Tetrahedron Lett.* **1986**, *27*, 3755−3758.

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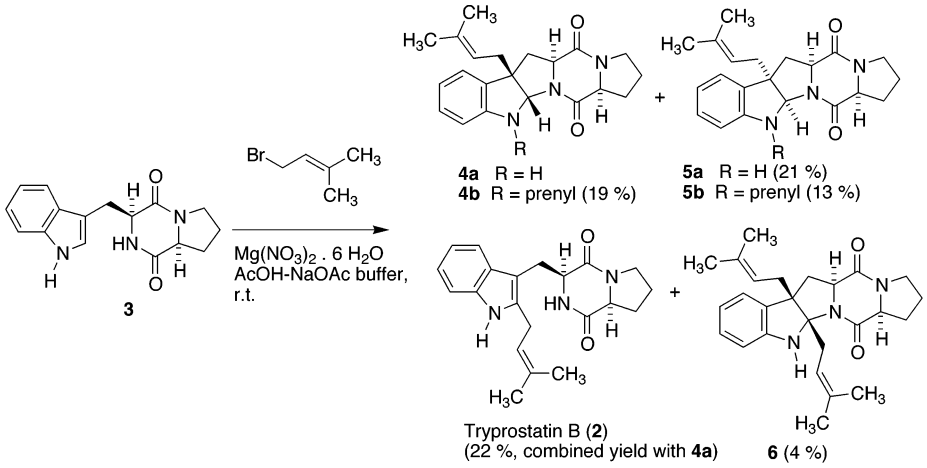
SCHEME 3. Tandem Prenylation−Cyclization of *cyclo*(L-Trp-L-Pro)



zinediones containing a tryptophan moiety[24] are well-known in the literature. On the other hand, the related reactions initiated by C-3 alkylation have been much less employed and do not normally give yields above 20% in the case of tryptophan and tryptamine derivatives.[25] To our knowledge, the cyclization in 15% yield of *cyclo*-(L-Trp-L-Ala) in the presence of prenyl bromide in acetic buffer by Casnati[26] is the only tandem prenylation−cyclization of a diketopiperazine containing a tryptophan subunit described in the literature, although the stereochemical outcome of the reaction was not studied.

Our work leading to tryprostatin B is summarized in Scheme 3. Slow addition of prenyl bromide to a solution of *cyclo*-(L-Trp-L-Pro) (**3**)[27] and magnesium nitrate[25b] in acetic buffer gave a mixture of diastereomers (**4a**, **5a**) arising from alkylation of the indole ring at C-3 and subsequent cyclization by nucleophilic attack of the neighboring piperazinedione nitrogen, together with their *N*-prenylated derivatives **4b** and **5b**. The reaction also gave 22% of an inseparable mixture of compound **4a** and tryprostatin B (**2**) and a trace (4%) of the diprenylated pentacyclic derivative **6**, arising from C_3-prenylation/cyclization of tryprostatin B. Use of prenyl iodide[28] as the electrophile led to increased yields of the *N*-prenyl derivatives: 6% of a **4a** + **2** mixture, 30% of **4b**, 6% of **5a**, and 29% of **5b**. Since even C-3-substituted indoles are normally alkylated at C-3 preferably to C-2, the formation of tryprostatin B observed by us can in principle be explained by acid-catalyzed rearrangement of **4a** or **5a** under the reaction conditions, although direct alkylation at C-2 cannot be completely excluded[29] and rearrangement of **5a** can be discarded, as shown by later results.

To trigger the desired rearrangement of the prenyl chain of compound **4a**, a number of acidic conditions were assayed, involving various trifluoroacetic acid concentrations and reaction times, leading always to mixtures of compound **7** and tryprostatin B (**2**). Since we were unable to prevent the addition of trifluoroacetic acid to the double bond of **2**, we decided to use a relatively concentrated acid solution and a long reaction time in order to ensure the isolation of **7** as the only reaction product and then transform it into the target compound by base-catalyzed elimination. Thus, the mixture of **4a** and **2** was exposed to 1:10 trifluoroacetic acid−dichloromethane for 20 h, giving a quantitative yield of trifluoroacetate ester **7**, which was characterized via its hydrolysis to **8**. Finally, exposure of compound **7** to triethylamine in methanol gave tryprostatin B in 96% yield (Scheme 4). We expected that compound **5a** could also be transformed into tryprostatin by a similar rearrangement of its prenyl chain. However, its treatment with several trifluoroacetic acid−dichloromethane mixtures gave only starting material, and forcing conditions (neat trifluoroacetic acid) led to compound **9** from addition of trifluoroacetic acid to the prenyl double bond, which, upon attempted silica gel chromatography, reverted to the starting material. Despite this drawback, we found that **5a** could also be transformed into tryprostatin B in 50% yield by prolonged treatment with ytterbium triflate in nitromethane, thus raising the overall yield of the route to 32% (Scheme 4). Ytterbium triflate is a well-known Lewis acid that promotes the addition of nucleophiles to aldehydes[30] and has also found application in the deprotection of prenyl ethers,[31] but we are unaware of any literature precedent on its use for promoting a rearrangement similar to the **5a** → **2** transformation.

(24) See ref 23a, and also: (a) Sammes, P. G.; Weedon, A. C. *J. Chem. Soc., Perkin Trans. 1* **1979**, 3048−3052. (b) Caballero, E.; Avendaño, C.; Menéndez, J. C. *Tetrahedron: Asymmetry* **1998**, *9*, 967−981. (c) Taniguchi, M.; Yamamoto, I.; Nakagawa, M.; Hino, T. *Chem. Pharm. Bull.* **1985**, *33*, 4783−4791.

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(26) Bocchi, V.; Casnati, G.; Marchelli, R. *Tetrahedron* **1978**, *34*, 929−932.

(27) Compound **3** has been prepared from *N*-Cbz-L-prolyl-L-tryptophan methyl ester (ref 8). Our procedure employing the *N*-Boc derivative is more convenient and can be found in Supporting Information.

(28) Prenyl iodide used in this experiment was prepared in 71% yield from prenyl alcohol and Olah's chlorotrimethylsilane/sodium iodide reagent. See: Olah, G. A.; Narang, S. B.; Gupta, B.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 1247−1251.

(29) (a) Joule, J. A.; Mills, K.; Smith, G. F. *Heterocyclic Chemistry*, 3rd ed.; Chapman & Hall: New York, 1995; p 303. (b) Casnati, G.; Dossena, A.; Pochini, A. *Tetrahedron Lett.* **1972**, 5277−5280.

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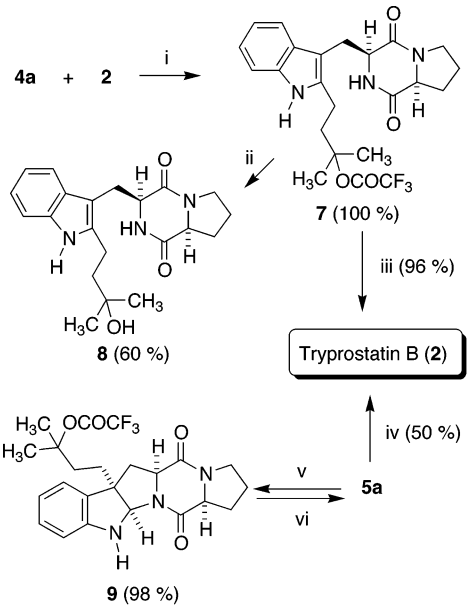
SCHEME 4. Final Stages of the Synthesis of Tryprostatin B[a]



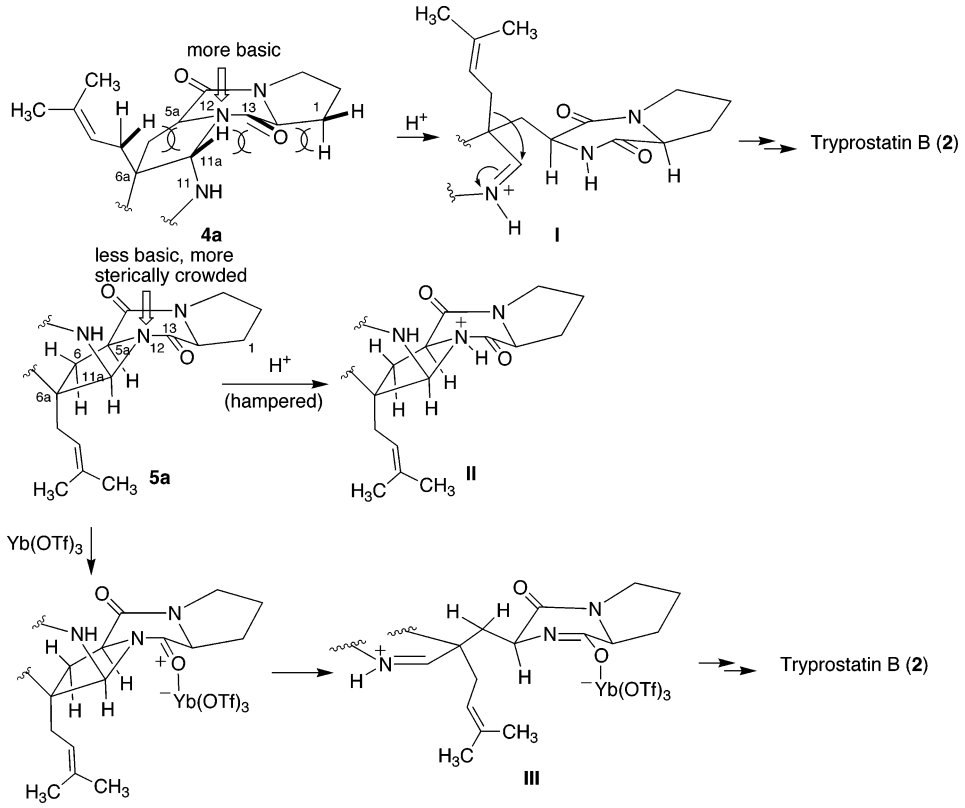
[a] Reagents and conditions: (i) 1:10 CF_3CO_2H, CH_2Cl_2, rt, 20 h; (ii) $NaHCO_3$, H_2O−THF, rt, 37 h; (iii) MeOH, Et_3N, rt, 60 h; (iv) $Yb(OTf)_3$, CH_3NO_2, reflux, 72 h; (v) CF_3CO_2H, rt, 2 h; (vi) SiO_2 chromatography (AcOEt).

The different behavior of compounds **4a** and **5a** toward acid-induced rearrangement can be ascribed to different basicities of their N-12 atoms and to the higher steric crowding of N-12 in compound **4a**. Steric compression between the C-13 carbonyl oxygen and one of the C_1−H bonds and the C_{11a}−H bond in **4a**, which are approximately parallel to C_{13}═O, can force the carbonyl group slightly out of conjugation with N-12, thus facilitating its protonation and subsequent rearrangement of the prenyl chain through indolenine **I**. This effect does not exist in compound **5a**, which is almost completely planar at the amide bond, as shown by the C_{11a}−N_{12}−C_{13}−O_{13} dihedral angle, which is −0.95° for **5a** and −3.05° for **4a** according to MM2 calculations, this difference being consistent with the one obtained using AM1 semiempirical calculations (ca. 3° lower for **5a**). Additionally, the higher steric crowding of the N-12 atom in compound **5a** may be the cause of a rate difference in its protonation with respect to compound **4a**, which undergoes the rearrangement (Scheme 5).

On the other hand, lanthanide(III) species are considered to be hard acids according to Pearson's HSAB classification and therefore show affinity toward hard bases such as oxygen donor ligands. Because of this oxophilicity and the high electron density of its C-13 carbonyl oxygen, compound **5a** would be expected to react with ytterbium triflate, giving the intermediate indolenine **III**, which would then undergo prenyl rearrangement to give tryprostatin B (Scheme 5). Because of the expected interference of the C_{5a}−H bond, this reaction would be expected to proceed slowly, which is in agreement with the experimental observations.

The preparation of tryprostatin B described above paved the way for the synthesis of analogues by simply replacing proline by other amino acids, although the practical realization of this modification might not be completely trivial since the presence of an additional NH group in the starting piperazinedione created a potential

SCHEME 5. Proposed Explanation for the Different Behaviors of Compounds 4a and 5a



SCHEME 6. Synthesis of the Alanine Analog of Tryprostatin B

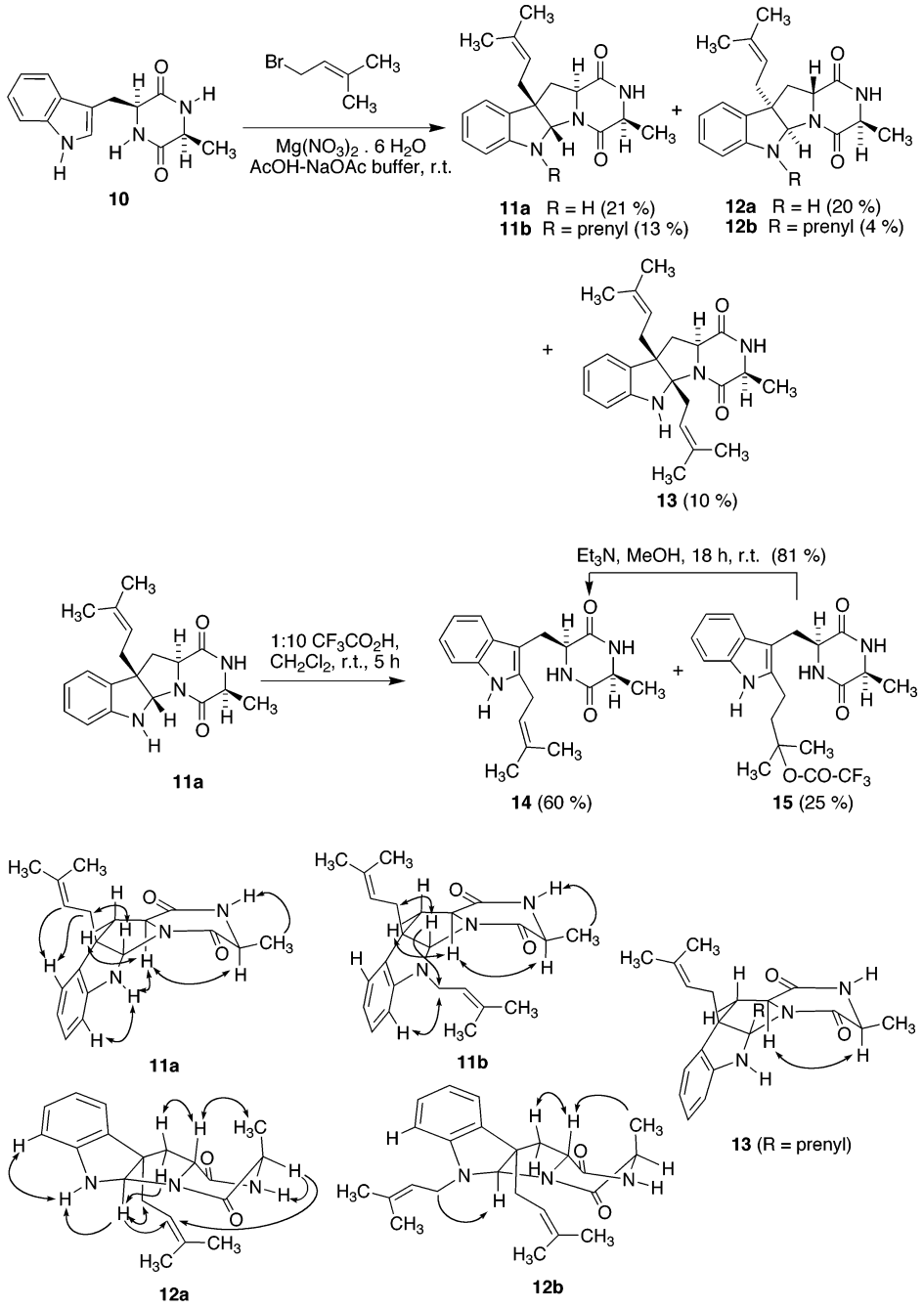


FIGURE 2. NOE effects in compounds **11**−**13**.

site for competitive alkylation in the first step of the synthesis. Fortunately, this problem did not arise, and we found that slow addition of prenyl bromide to a solution of *cyclo*-(L-Trp-L-Ala) (**10**) gave a mixture of diastereomers (**11a**, **12a**), similarly formed by alkylation of the indole ring at C-3 and tandem cyclization by nucleophilic attack of the neighboring piperazinedione nitrogen. Their *N*-prenylated derivatives **11b** and **12b**, respectively, as well as compound **13**, prenylated at both atoms of the indoline-pyrrolidine fusion bond, were also obtained. Treatment of compound **11a** with dilute trifluoroacetic acid gave the desired tryprostatin analogue **14** as the major product (60% yield), together with 25% of the trifluoroacetate **15**, which could be transformed into **14** in 81% yield by reaction with triethylamine in methanol (Scheme 6).

The stereochemical assignments of the intermediate tetracyclic derivatives in the alanine series (compounds **11**−**13**) were based on the NOE experiments summarized in Figure 2. We assume that epimerization of the Trp stereocenter in **12a** takes place after cyclization and is prompted by the considerable steric crowding of the nonisolated compound **IV** due to the interaction between the C_{5a}−H, C_{11}−H, and C_{10b}−prenyl substituents, which is partly relieved by its transformation into **12**. This steric crowding does not exist in compounds **11**, which therefore

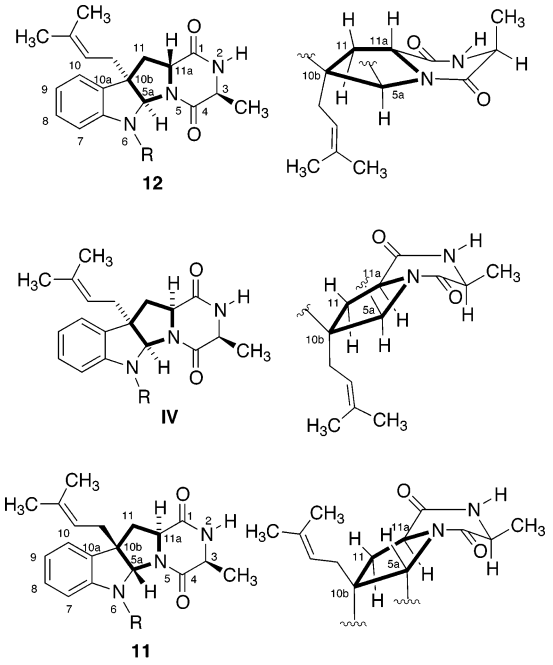


FIGURE 3. Comparison between the structures of compounds **11** and **12** and their nonisolated epimer at C-11a (**IV**).

do not epimerize under acidic conditions (e.g., dilute CF_3-CO_2H in the transformation of **11a** into **14** and **15**). Both results, namely, the observed epimerization at the tryptophan stereocenter of **12** and the absence of epimerization in **11**, are consistent with our previous findings on the tandem protonation−cyclization reactions of *cyclo*-(Trp-Ala)[24b] (Figure 3).

On the other hand, the pentacyclic derivatives related to tryprostatin B (compounds **4**−**6**) were not equally amenable to NOE studies, and we had to resort to comparisons of some key ^{1}H NMR chemical shifts and multiplicities to establish their stereochemistries. In the alanine series, the H-11 signals appear as two dd at ca. 2.60 and 2.30 ppm for compounds with a trans relationship between the substituents at positions 5a−10b and the H-3 proton (compounds **11** and **13**) but as multiplets centered at ca. 2.30 ppm for their diastereomers where this relationship is cis (compounds **12**). Another significant difference can be found in the chemical shift of the bridgehead proton H-5a, which is influenced by prenylation at N-6 in the case of compounds **12** (5.29 ppm for **12a** and 5.51 ppm for **12b**), but not in the case of compounds **11** and **13**, where it is observed at 5.40 ppm in all cases. Spectral data of compounds **4** and **5** fit very well into these criteria, allowing assignment of their stereochemistries. Epimerization at the tryptophan stereocenter was considered to be dubious because, according to the literature, this epimerization does not take place in the protonation−cyclization of *cyclo*-(L-Trp-L-Pro).[24c] Although the key NOE effects of the tryptophan H-5a proton were ambiguous because the signals due to one of the H-6 protons and the prenyl methylene are overlapped, the fact that **5a** can be rearranged to tryprostatin B confirms the proposed configuration at H-5a. The difference between the alanine and proline series in terms of epimerization of the tryptophan stereocenter can be attributed to the fact that the nonisolated compound

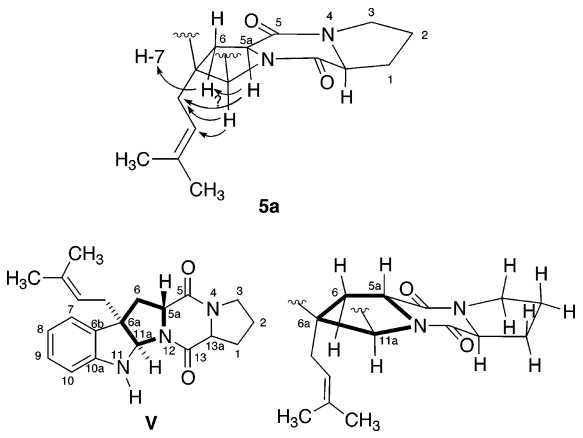


FIGURE 4. NOE effects in compound **5a** and structure of compound **V**, the nonisolated 5a-epimer of **5a**.

V (5a-epi-**5a**) would be forced to have its piperazinedione ring in a chair conformation (Figure 4), which does not happen in its alanine analogue.

As a final note, we believe that the tryprostatin B synthesis described in this work has implications regarding the yet unknown biosynthetic pathway of the tryprostatins[32] in that it provides an alternative to the acid-catalyzed rearrangement of 1-allylindole derivatives, which has been shown to give product mixtures containing variable amounts of 2-allylindoles in work aimed at clarifying the biosynthetic pathways of the echinulins, a related group of prenylindole alkaloids.[33]

Experimental Section

Tandem Prenylation−Cyclization of *cyclo*(L-Trp-L-Pro) (3). To a vigorously stirred solution of *cyclo*(L-Trp-L-Pro) **3**[27] (350 mg, 1.24 mmol) and magnesium nitrate hexahydrate (1.59 g, 5 equiv) in 50 mL of aqueous acetic acid−sodium acetate buffer (pH 2.9, prepared from 8 g of sodium acetate, 20 mL of water, and 100 mL of acetic acid) was slowly added at room-temperature prenyl bromide (0.85 mL, 6 equiv) over 20 h, via syringe pump, under an argon atmosphere. The solution was neutralized with solid Na_2CO_3 and extracted with $CHCl_3$ (10 × 10 mL), filtering off any inorganic precipitate formed during the extraction. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated. The residue was evaporated to dryness and chromatographed on silica gel, eluting with 1:1 petroleum ether−ethyl acetate, yielding (in order of elution) 31 mg (4%) of (5a*S*,6a*S*,11a*S*,-13a*S*)-6a,11a-diprenyl-2,3,5,5a,6,6a,11,11a,13,13a-decahydro-1*H*-pyrrolo[1″,2″-4′,5′]pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-5,13-dione (**6**), 67 mg (13%) of (5a*S*,6a*R*,11a*R*,13a*S*)-6a,11-diprenyl-2,3,5,5a,6,6a,11,11a,13,13a-decahydro-1*H*-pyrrolo[1″,2″-4′,5′]pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-5,13-dione (**5b**), 96 mg (19%) of (5a*S*,6*S*,11a*S*,13a*S*)-6a,11-diprenyl-2,3,5,5a,6,6a,-11,11a,13,13a-decahydro-1*H*-pyrrolo[1″,2″-4′,5′]pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-5,13-dione (**4b**), 93 mg (21%) of (5a*S*,-6a*R*,11a*R*,13a*S*)-6a-prenyl-2,3,5,5a,6,6a,11,11a,13,13a-decahydro-1*H*-pyrrolo[1″,2″-4′,5′]pyrazino[2′,1′-5,1]pyrrolo[2,3-

(32) For a review of the biosynthesis of prenylated alkaloids derived from tryptophan, see: Williams, R. M.; Stocking, E. M.; Sanz-Cervera, J. F. *Top. Curr. Chem.* **2000**, *209*, 98−173.

(33) (a) Casnati, G.; Marchelli, R.; Pochini, A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 754−757. (b) Inada, S.; Nagai, K.; Takayanagi, Y.; Okazaki, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 833−834. (c) Sammes, P. G.; Weedon, A. C. *J. Chem. Soc., Perkin Trans. 1* **1979**, 3053−3059. (d) Grundon, M. F.; Hamblin, M. R.; Harrison, D. M.; Logue, J. N. D.; Maguire, M.; McGrath, J. A. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1294−1298. (e) See also ref 18.

b]indole-5,13-dione (**5a**), and 123 mg (44%) of a 1:1 inseparable mixture of compound **4a**[34] and tryprostatin B (**2**).

Data for 4b. Pale yellow oil. $[\alpha]^{D}_{25} = +1.60$ ($c = 1.35$, $CHCl_3$). Anal. Calcd for $C_{26}H_{33}N_3O_2$, M = 419: C, 74.43; H, 7.93; N, 10.02. Found: C, 74.45; H, 7.87; N, 9.94. For spectral data, see Supporting Information.

Data for 5a. Off-white solid. Mp 65−69 °C. $[\alpha]^{D}_{25} = -236.8$ ($c = 0.72$, $CHCl_3$). Anal. Calcd for $C_{21}H_{25}N_3O_2$, M = 351: C, 71.77; H, 7.17; N, 11.96. Found: C, 71.71; H, 6.97; N, 11.99. For spectral data, see Supporting Information.

Data for 5b. Pale yellow oil. $[\alpha]^{D}_{25} = -197.8$ ($c = 0.69$, $CHCl_3$). For spectral data, see Supporting Information.

Data for 6. Off-white solid. Mp 63−65 °C. $[\alpha]^{D}_{25} = -210.1$ ($c = 0.69$, $CHCl_3$). Anal. Calcd for $C_{26}H_{33}N_3O_2$, M = 419: C, 74.46; H, 7.88; N, 10.02. Found: C, 74.79; H, 7.68; N, 9.81. For spectral data, see Supporting Information.

Transformation of the 4a + 2 Mixture into Pure Tryprostatin B. To a solution of the equimolecular mixture of **4a** and **2** (27 mg, 0.077 mmol) in CH_2Cl_2 (5 mL) was added CF_3CO_2H (0.5 mL). The solution was stirred at room temperature for 20 h, under an argon atmosphere, and then it was poured onto a cooled (5 °C), vigorously stirred mixture of 20% aqueous Na_2CO_3 (7 mL) and CH_2Cl_2 (7 mL). After further extraction with CH_2Cl_2 (7 × 5 mL), the combined organic layers were dried over anhydrous Na_2SO_4 and evaporated, the residue being identified by ^{1}H NMR and ^{19}F NMR as (3*S*,8a*S*)-3-[2′-(3″,3″-dimethy-3″-trifluoroacetoxy)propyl-3′-indolylmethyl]-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**7**).[35] A solution of the crude compound **7** in methanol (2 mL) and triethylamine (2 mL) was stirred at room temperature for 60 h under an argon atmosphere. The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 and washed with a saturated aqueous solution of NH_4Cl (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated, yielding 25 mg (96%) of tryprostatin B (**2**). Off-white solid. Mp 102−104 °C; lit.[4a] 102−105 °C. $[\alpha]^{D}_{25} = -83.3$ ($c = 0.03$, $CHCl_3$); lit[4a] $[\alpha]^{D}_{25} = -71.1$ ($c = 0.63$, $CHCl_3$). IR (NaCl): 3284; 1665 cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.91 (br s, 1H); 7.48 (d, 1H, $J = 7.4$ Hz); 7.32 (d, 1H, $J = 7.8$ Hz); 7.12 (m, 2H); 5.63 (br s, 1H); 5.31 (m, 1H); 4.37 (m, 1H); 4.06 (m, 1H); 3.66 (m, 3H); 3.48 (app d, 1H, $J = 7.6$ Hz); 2.95 (dd, 1H, $J = 15.0$ and 11.5 Hz); 2.34 (m, 1H); 2.03 (m, 3H); 1.79 (s, 3H); 1.76 (s, 3H).^{13}C NMR ($CDCl_3$, 63 MHz): δ 169.5; 165.9; 136.6; 135.8; 135.5; 128.1; 121.9; 120.0; 119.9; 117.8; 110.9; 104.7; 59.4; 54.7; 45.5; 28.4; 25.9; 25.7; 25.2; 22.7; 18.3. MS, *m*/*z* (%): 351 (13.2, M^+); 282 (8.7, M^+ − prenyl); 198 (100, $M^+ - C_7H_9N_2O_2$). HRMS: calcd for $C_{21}H_{25}N_3O_2$ (M^+) 351.1947, found 351.1949; calcd for $C_{16}H_{16}N_3O_2$ (M^+ − prenyl) 282.1243, found 282.1242; calcd for $C_{14}H_{16}N$ ($M^+ - C_7H_9N_2O_2$) 198.1283, found 198.1279.

Transformation of 5a into Tryprostatin B. A solution of compound **5a** (50 mg, 0.14 mmol) in nitromethane (2 mL) was treated with ytterbium triflate (9 mg, 0.014 mmol) and refluxed in an oil bath at 115 °C for 72 h. The reaction mixture was diluted with water (5 mL) and extracted with $CHCl_3$ (5 × 10 mL). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated, and the residue was chromatographed on silica gel, eluting with ethyl acetate. The yield of tryprostatin B (**2**) was 25 mg (50%).

Hydrolysis of Compound 7. To a solution of **7** (8.5 mg, 0.024 mmol) in THF (1 mL) was added a solution of $NaHCO_3$ (44.75 mg, 22 equiv) in water (1 mL). The solution was stirred at room temperature under an argon atmosphere for 37 h and extracted with $CHCl_3$ (7 × 5 mL). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel, eluting with 4:1 AcOEt−MeOH, yielding 4 mg (60%) of (3*S*,8a*S*)-3-(2′-(3″-hydroxy-3″,3″-dimethylpropyl)-3′-indolylmethyl)-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**8**). Mp 90−92 °C. $[\alpha]^{D}_{25} = +35.0$ ($c = 0.08$, $CHCl_3$). IR (NaCl): 3349; 1663 cm^{-1}. HRMS: calcd for $C_{21}H_{27}N_3O_3$ (M^+) 369.2052, found 369.2044; calcd for $C_{14}H_{18}NO$ ($M^+ - C_7H_9N_2O_2$), 216.1388, found 216.1386; calcd for $C_{14}H_{16}N$ ($M^+ - C_7H_{11}N_2O_3$) 198.1283, found 198.1281.

Reaction of Compound 5a with Trifluoroacetic Acid. A solution of **5a** (27 mg, 0.077 mmol) in neat trifluoroacetic acid (1 mL) was stirred at room temperature for 2 h and poured onto a cooled (0 °C), stirred mixture of 20% aqueous Na_2CO_3 (5 mL) and CH_2Cl_2 (5 mL). The aqueous phase was further extracted with CH_2Cl_2 (7 × 5 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, giving compound **9**[36] (35 mg, 98%). Attempted silica gel chromatography, eluting with ethyl acetate, gave the starting material **5a**.

Tandem Prenylation−Cyclization of *cyclo*(L-Trp-L-Ala) (10). To a vigorously stirred solution of *cyclo*(L-Trp-L-Ala) (**10**)[37] (500 mg, 1.95 mmol) and magnesium nitrate hexahydrate (2.5 g, 5 equiv) in 50 mL of acetic acid−sodium acetate buffer (pH = 2.9) was slowly added at room-temperature prenyl bromide (1.5 mL, 6 equiv) over 12 h, via syringe pump, under an argon atmosphere. The solution was neutralized with solid Na_2CO_3 and extracted with $CHCl_3$ (10 × 10 mL), filtering off any inorganic precipitate formed during the extraction. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated. The residue was evaporated to dryness and chromatographed on silica gel, eluting with 1:1 petroleum ether−ethyl acetate, yielding 132 mg (21%) of (3*S*,5a*S*,10b*S*,-11a*S*)-3-methyl-10b-prenyl-1,3,4,5a,6,10b,11,11a-octahydro-2*H*-pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione (**11a**), 129 mg (20%) of its (10b*R*,11a*R*)-diastereomer (**12a**), 101 mg (13%) of (3*S*,5a*S*,10b*S*,11a*S*)-3-methyl-6,10b-diprenyl-1,3,4,5a,6,10b,-11,11a-octahydro-2*H*-pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione (**11b**), 29 mg (4%) of its (10b*R*,11a*R*)-diastereomer (**12b**), and 78 mg (10%) of (3*S*,5a*S*,10b*S*,11a*S*)-3-methyl-5a,-10b-diprenyl-1,3,4,5a,6,10b,11,11a-octahydro-2*H*-pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione (**13**).

Data for 11a. White solid. Mp 187−188 °C. $[\alpha]^{D}_{25} = +122.3$ ($c = 0.13$, $CHCl_3$). Anal. Calcd for $C_{19}H_{23}N_3O_2$, M = 325: C, 70.15; H, 7.08; N, 12.92. Found: C, 69.93; H, 6.88; N, 13.09. For spectral data, see Supporting Information.

Data for 11b. Off-white solid. Mp 60−61 °C. $[\alpha]^{D}_{25} = -40.3$ ($c = 0.66$, $CHCl_3$). Anal. Calcd for $C_{24}H_{31}N_3O_2$, M = 393: C, 73.28; H, 7.89; N, 10.69. Found: C, 73.00; H, 7.97; N, 10.42. For spectral data, see Supporting Information.

Data for 12a. Off-white solid. Mp 70−72 °C. $[\alpha]^{D}_{25} = -353.0$ ($c = 0.20$, $CHCl_3$). Anal. Calcd for $C_{19}H_{23}N_3O_2$, M = 325: C, 70.15; H, 7.08; N, 12.92. Found: C, 69.91; H, 6.93; N, 12.87. For spectral data, see Supporting Information.

Data for 12b. Pale yellow oil. $[\alpha]^{D}_{25} = -220.8$ ($c = 0.06$, $CHCl_3$). Anal. Calcd. for $C_{24}H_{31}N_3O_2$, M = 393: C, 73.28; H, 7.89; N, 10.69. Found: C, 73.44; H, 7.53; N, 10.86. For spectral data, see Supporting Information.

Data for 13. Off-white solid. Mp 65−64 °C. $[\alpha]^{D}_{25} = -170.9$ ($c = 0.43$, $CHCl_3$). Anal. Calcd for $C_{24}H_{31}N_3O_2$, M = 393: C, 73.28; H, 7.89; N, 10.69. Found: C, 73.58; H, 7.60; N, 10.50.

(34) ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.07 (m, 2H); 6.75 (td, 1H, J = 7.4 and 0.8 Hz); 6.58 (d, 1H, $J = 7.7$ Hz); 5.42 (s, 1H); 5.10 (t, 1H, J = 7.9 Hz); 4.36 (t, 1H, $J = 8.6$ Hz); 4.14 (m, 1H); 3.48 (m, 2H); 2.50 (m, 4H); 2.32 (m, 1H); 2.22−1.87 (m, 3H); 1.69 (s, 3H); 1.62 (s, 3H). ^{13}C NMR ($CDCl_3$, 63 MHz): δ 168.1; 166.0; 147.5; 135.5; 132.8; 128.4; 123.0; 119.3; 118.9; 109.4; 80.9; 60.7; 59.8; 56.2; 45.2; 38.7; 35.4; 27.8; 26.1; 23.3; 18.1.

(35) ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.05 (br s, 1H); 7.48 (d, 1H, J = 7.6 Hz); 7.33 (d, 1H, $J = 7.3$ Hz); 7.12 (m, 2H); 5.63 (br s, 1H); 4, 38 (m, 1H); 4.08 (m, 1H); 3.64 (m, 3H); 2.95 (m, 1H); 2.82 (m, 2H); 2.35 (m, 1H); 2.20 (m, 2H); 2.03 (m, 3H); 1.63 (s, 3H); 1.62 (s, 3H) ppm. ^{19}F NMR ($CDCl_3$): δ −76.00 (s).

(36) ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.10 (m, 2H); 6.82 (t, 1H, J = 7.4 Hz); 6.63 (d, 1H, $J = 7.7$ Hz); 5.25 (s, 1H); 4.09 (m, 2H); 3.55 (m, 2H); 2.72 (dd, 1H, $J = 13.8$ and 8.1 Hz); 2.32 (m, 3H); 2.24−1.54 (m, 8H); 1.47 (s, 3H); 1.25 (s, 3H). ^{19}F NMR ($CDCl_3$): δ −76.1 (s).

(37) Compound **10** has been isolated from natural sources. See, for instance: Hamasaki, T.; Nagayama, K.; Hatsuda, Y. *Agric. Biol. Chem.* **1976**, *40*, 2487. For its preparation, see ref 24b.

Reaction of Compound 11a with Trifluoroacetic Acid. To a solution of compound **11a** (50 mg, 0.154 mmol) in CH_2Cl_2 (1.5 mL) was added trifluoroacetic acid (1 mL). The solution was stirred under an argon atmosphere for 4.5 h and poured onto a vigorously stirred biphasic system formed by 20% aqueous Na_2CO_3 (7 mL) and CH_2Cl_2 (5 mL), kept in a bath at 4 °C. The aqueous layer was extracted with CH_2Cl_2 (5 × 5 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel, eluting with ethyl acetate, giving 30 mg (60%) of (3*S*,6*S*)-3-(2′-prenyl-3′-indolylmethyl)-6-methyl-2,5-piperazinedione **14**, the alanine analogue of tryprostatin B, as a white solid, and 17 mg (25%) of (3*S*,6*S*)-3-[2′-(3″,3″-dimethyl-3″-trifluoroacetoxy)propyl-3′-indolylmethyl]-6-methyl-2,5-piperazinedione (**15**), as a pale yellow solid.

Data for 14. White solid. Mp 240−242 °C. $[\alpha]^{D}_{25} = -21.1$ ($c = 0.09$, $CHCl_3$). HRMS: calcd for $C_{19}H_{23}N_3O_2$ (M^+) 325,1790, found 325.1789; calcd for $C_{14}H_{16}N$ ($M^+ - C_5H_7N_2O_2$) 198.1283, found 198.1278. For spectral data, see Supporting Information.

Data for 15. Pale yellow solid. Mp 273−275 °C. $[\alpha]^{D}_{25} = -7.4$ ($c = 0.27$, EtOH). Anal. Calcd for $C_{29}H_{24}F_3N_3O_4$, M = 439: C, 57.40; H, 5.47; N, 9.57. Found: C, 57.37; H, 5.70; N, 9.59. For spectral data, see Supplementary Information.

Transformation of Compound 15 into 14. To a solution of compound **15** (10 mg, 0.03 mmol) in methanol (2 mL) was added triethylamine (2 mL). The solution was stirred at room temperature, under an argon atmosphere, for 18 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (10 mL) and washed with a saturated aqueous solution of NH_4Cl (5 mL). The organic layer was dried over Na_2SO_4 and evaporated, yielding 6 mg (81%) of compound **14**.

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Supporting Information Available: Experimental details of the synthesis of starting materials, spectral data, and spectra of representative compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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