Enediynes as Antitumor Compounds: Synthesis of Tetrahydropyridine Derivatives

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Enediyne anticancer antibiotics are a rapidly emerging class of compounds derived from natural sources. Many synthetic approaches for the construction of simpler compounds containing this pharmacophore have recently been published. Most of these approaches use the quinoline ring as the heterocyclic moiety . In this paper, we describe a synthetic route for the preparation of enediyne systems **14** and **15**, which include in their structure a tetrahydropyridine ring and are related to dynemicin A. Their stereochemistries were determined using NOESY and COSY-NMR. Finally, compounds derived from linkage to intercalating carriers, such as a fluorene ring to enediyne **15** or a naphthalimide ring to acyclic enediyne **6**, were prepared.

Introduction

A new class of novel bicyclic antibiotics possessing an unprecedented (*Z*)-enediyne unit has recently been discovered and is one of the most potent antitumor agents identified thus far. This family of compounds includes calicheamicin $\gamma_1{}^I$, esperamicin A_1, neocarzinostatin chromophore, and dynemicin A.[1] They have aroused considerable interest due to their unusual features, and exert their biological action by virtue of their ability to cleave DNA. These antibiotics, after an appropriate triggering event such as bioreduction, can undergo Bergman-type cyclization,[2] except for neocarzinostatin, which undergoes Myers-type cyclization, to form a benzenoid diradical. These highly reactive radicals are capable of abstracting hydrogen atoms from DNA backbone, which initiates DNA strand cleavage. However, the toxicity shown by these compounds prevents their clinical application.[3] As a consequence, many synthetic approaches for the construction of simpler compounds containing the enediyne pharmacophore have recently been published.[4]

Dynemicin A can be considered a hybrid between two types of well-known antitumor agents: anthracycline and enediyne antibiotics. However, in most models described, the enediyne system is linked to quinoline derivatives.[5] We previously reported[6] the synthesis of enediyne derivatives in which a tetrahydropyridine system is present. This was the first reported model with this heterocyclic moiety. We describe here the detailed synthetic route for the preparation of enediyne models **14** and **15**, as well as the full assignment of their stereochemistries. We also report the synthesis of compounds derived from the linkage of **6** and **19**, the former of which is a synthetic intermediate in the synthesis of **14** and **15**, to different intercalators such as fluorene and naphthalimide, whose main representatives as antitumor agents are Tilorone[7] and Amonafide.[8] We used this approach to compare the cytostatic activities of acyclic and cyclic enediynes.

Results and Discussion

The synthetic route first proposed for construction of the targeted compound **8** is outlined in Scheme 1.

Protection of the hydroxyl group of commercially available 2-(hydroxyethyl)pyridine (**1**) as *tert*-butyldimethylsilyl ether **2** was easily accomplished in 95% yield. Based on the methodology described by Yamaguchi,[9] treatment of **2** with ethyl chloroformate and [(trimethylsilyl)ethynyl]magnesium bromide at 0 °C led to the 1,2-addition product **3** in 44% yield. Deprotection of 1,2-dihydropyridine **3** was carried out by treatment with hydrofluoric acid in acetonitrile to give alcohol **4** in 60% yield. However, treatment of **4** under (trimethylsilyl)-acetylene deprotection conditions, (K_2CO_3/MeOH) did not lead to the desired compound **5**, and instead gave the unexpected product **9**. The absence of a strong absorption band in the IR spectrum typical of an hydroxyl group and of signals corresponding to the ethyl group of the ethoxycarbonyl functionality in the ^{1}H−NMR spectrum indicated that the compound obtained did not correspond to the desired **5**. A detailed analysis of the ^{1}H-^{13}C-NMR (COSY) and mass spectra and an elemental analysis provided support for the assignment of structure **9** to the compound isolated. The formation of cyclic lactone **9** could be explained by an intramolecular esterification reaction. To our knowledge, these reactions are unusual, and only one example of this type has been published.[10]

These results forced us to design a new route, depicted in Scheme 2, for the synthesis of compound **8**, which

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[1] For a review see: (a) Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30,* 1387. (b) Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256,* 1172. (c) Nicolaou, K. C.; Smith, A. L.; Yue E. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90,* 5881 and references therein.

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[4] (a) Takahashi, T.; Tanaka, H.; Hirai, Y.; Doi, T.; Yamada, H.; Shiraki, T.; Sugiura, Y. *Angew. Chem., Int. Ed. Engl.* **1993**, *32,* 1657. (b) Yoon, T.; Shair, M. D.; Danishefsky, S. J.; Shulte, G. K. *J. Org. Chem.* **1994**, *59,* 3752. (c) Maier, M. E.; Abel, U. *Synlett* **1995**, 38.

[5] (a) Porco J. A., Jr.; Schoenen, F. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112,* 7410. (b) Wender, P. A.; Zercher, C. K.; Beckhman, S.; Haubold, E.-M. *J. Org. Chem.* **1993**, *58,* 5867. (c) Nishikawa, T.; Ino, A.; Isobe, M. *Tetrahedron* **1994**, *50,* 1449.

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[9] Yamaguchi, R.; Nakazono,Y.; Kawanishi, M. *Tetrahedron Lett.* **1983**, *24,* 1801.

[10] Lazar, L.; Fueloep, F.; Dombi, G.; Bernath, G.; Argay, G.; Kalman, A. *Hung. Magy. Kem. Foly.* **1990**, *96,* 349.

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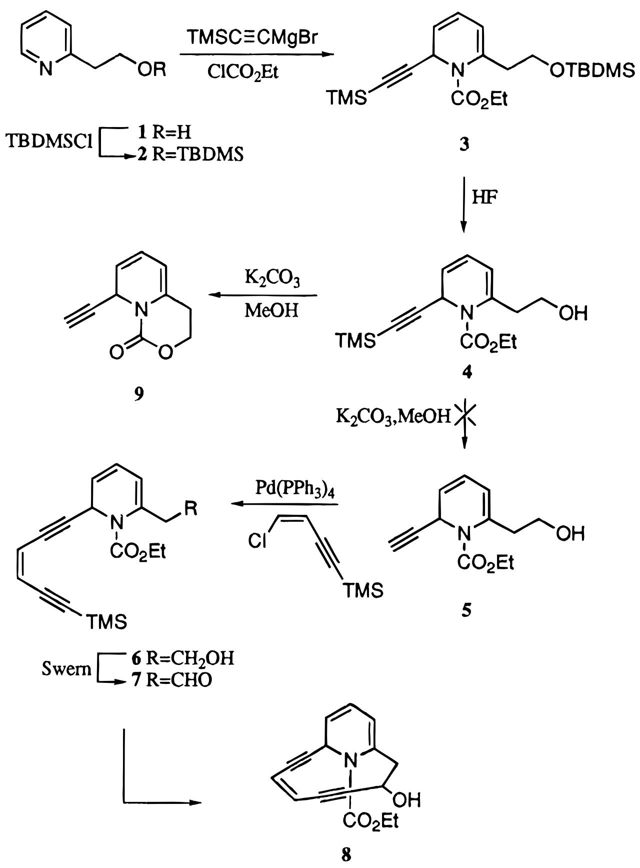
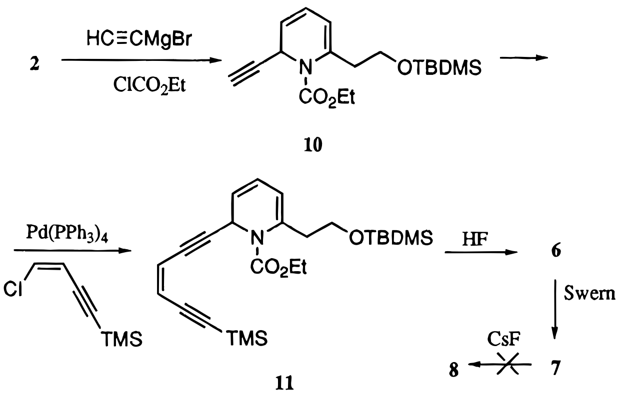
Scheme 1



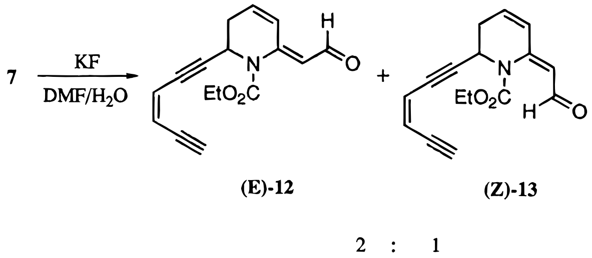
Scheme 2



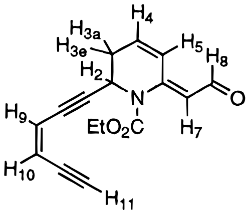
avoids the alkyne deprotection step in the presence of an hydroxyethyl group at position 6.

In this case, the deprotected ethynyl group was directly introduced into the pyridine ring using commercial ethynylmagnesium bromide and ethyl chloroformate following the same method as in **3**, to give carbamate **10** in 62% yield. Coupling of the terminal alkyne **10** with (*Z*)-1-chloro-4-(trimethylsilyl)-1-buten-3-yne[11] was accomplished via Pd(0)–Cu(I) catalysis[12] to provide enediyne **11** in 63% yield. Deprotection of the hydroxyl group of **11** by treatment with hydrofluoric acid at −20°C gave rise to alcohol **6** (85%), the oxidation of which under Swern conditions led to aldehyde **7** in 45% yield. This low yield may be attributed to the instability of **7**. The following step was intended to be a one-pot deprotection-cyclization reaction of aldehyde **7**. This was attempted under the conditions described by Wender[13] (CsF/CH_3CN) and Isobe[14] (CsF/CH_3CN/18-crown-6), but only decomposition products were observed in both cases. On the basis of these results, we decided to perform the reaction in two steps, the first involving deprotection of the terminal alkyne, and the second involving cyclization to the 10-membered ring. Thus, aldehyde **7** was reacted with KF in DMF/H_2O to give a 2:1 mixture, based on ^1H-NMR, of two deprotected isomeric aldehydes **12** and **13**.

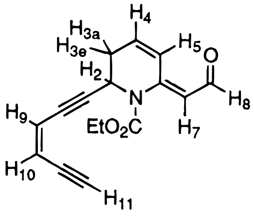
Scheme 3



Chromatographic separation of these isomers and examination of their $^1H-^1H$ COSY and NOESY spectra led us to conclude that the result of the reaction was a mixture of (*E*)-**12** and (*Z*)-**13** isomers. To determine the *Z* or *E* configuration of the exocyclic double bond of each isomer, 2D-NOESY spectra were obtained.

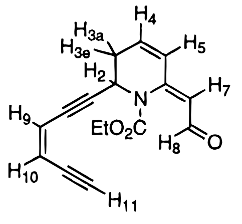


s-trans-(E)-12

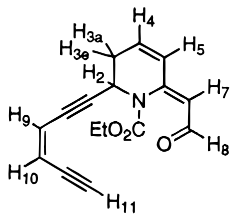


s-cis-(E)-12

In (*E*)-**12**, the presence of a very strong cross peak H_5/H_8 indicated that the double bond possesses an *E* configuration. In addition, since a cross peak H_7/H_8 is also observed, both the s-cis and s-trans conformations are present.



s-trans-(Z)-13



s-cis-(Z)-13

For isomer (*Z*)-**13**, the *Z* configuration of the exocyclic double bond was confirmed by the presence of a cross peak H_5/H_7 in the 2D-NOESY spectrum. This result was warranted by the presence of cross peaks H_7/H_8 and H_8/-CH_2O-, which are indicative, as in the other isomer, of the existence of both the s-cis and s-trans conformations.

However, despite these data, the position of the endocyclic double bond in (*Z*)-**13** was not clear, due to overlapping of the signals corresponding to H_2 and H_7,

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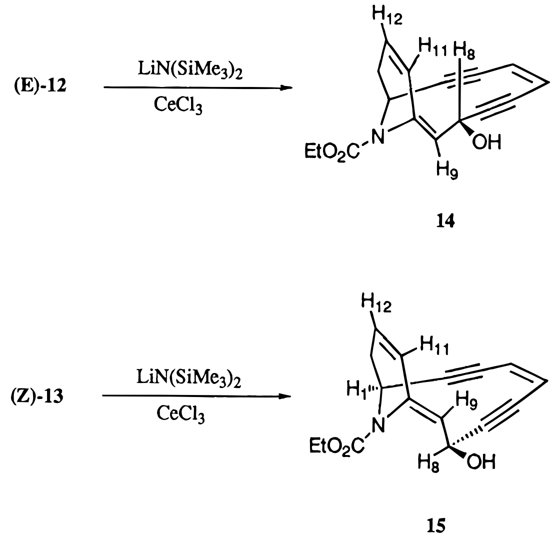
[11] Kende, A. S.; Smith, C. A. *Tetrahedron Lett.* **1988**, *29*, 4217.

[12] (a) Stephans, R. D.; Castro, C. E. *J. Org. Chem.* **1963**, *28*, 3313. (b) Ratovelomanana, V.; Linstrumelle, G. *Tetrahedron Lett.* **1981**, *22*, 315. (c) Guillerm, D.; Linstrumelle, G. *Tetrahedron Lett.* **1985**, *26*, 3811.

[13] Wender, P. A.; Zercher, C. K. *J. Am. Chem. Soc.* **1991**, *113*, 2311.

[14] Nishikawa, T.; Ino, A.; Isobe, M.; Goto, T. *Chem. Lett.* **1991**, 1271.

Scheme 4

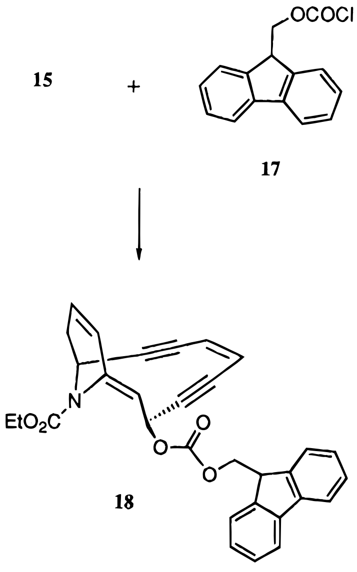


which appeared coupled with the CH_2 of the ring in the ^{1}H-NMR spectrum. In addition, the presence of NOE between the CH_2 and the multiplet which includes both H_2 and H_7, led us think that the double bond could be at position C_3-C_4 instead of C_4-C_5. We first tried to solve this ambiguity by obtaining the ^{1}H-NMR spectrum in different solvents, such as acetone, methanol, or benzene, but the signals continued to overlap. However, if the double bond were at C_3-C_4, the NOESY spectrum should show a cross peak H_2/H_3, and the absence of such a peak supports structure (*Z*)-**13**. It also seems reasonable that the compound obtained in this reaction should be that with the maximum conjugation between double bonds. In summary, we did not possess enough data regarding isomer (*Z*)-**13** to unambiguously determine the position of the endocyclic double bond.[15] The fact that aldehydes (*E*)-**12** and (*Z*)-**13** were obtained in a relative proportion of 2:1 can be explained by steric hindrance between the formyl and ethoxycarbonyl groups in the latter.

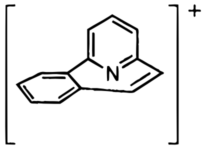
Cyclization of both aldehydes (Scheme 4) was first attempted by treatment with LDA/THF at −78°C, but only the starting material was recovered after a usual workup. Cyclization was finally achieved by addition of 1.2 equiv of $KN(SiMe_3)_2$ to the corresponding aldehyde in THF at −78°C. The resulting compounds **14** and **15** were obtained in yields of 10 and 30%, respectively, and were fully characterized spectroscopically. Cyclization yields for compounds **14** and **15** were improved when $LiN(SiMe_3)_2$ was used in the presence of $CeCl_3$[16] (35 and 51%, respectively). It should be pointed out that **14** and **15** are quite unstable at room temperature.

To determine the position of the hydroxyl group in the cyclic compounds, 2D-NOESY spectra were obtained. With regard to **14**, a strong cross peak H_8/H_{11} confirmed the equatorial position of the OH. For isomer **15**, the use of selective decoupling and 2D-NOESY techniques established the configuration of C_8 and unambiguously confirmed the position of the endocyclic double bond. The presence of a very weak cross peak H_8/H_9 indicated that the hydroxyl group is in the equatorial position. In the ^{1}H-NMR spectrum of **15**, the signals for H_1 and H_9 can be clearly identified and separated. Therefore, the presence of very strong peaks H_9/H_{11} and H_{11}/H_{12} confirmed that the endocyclic double bond is at $C_{11}-C_{12}$. These data were used to corroborate the structure of the precursor aldehyde (*Z*)-**13**.

Scheme 5



In the mass spectra of **14** and **15** during the electronic impact process, the presence of a peak (*m*/*e* 178), which was assigned to the fragment ion **16**, prompted us to consider the possibility that these compounds undergo Bergman cycloaromatization. However, their instability prevented cycloaromatization experiments to isolate the Bergman products.



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In vitro cytotoxicity assays were performed with **14** and **15**, which were stored under dry ice until they were tested. However, the IC_{50} results (10^{-5} M) were not good enough for further studies.

As noted above, some enediyne antibiotics, such as dynemicin A or neocarzinostatin chromophore, include in their structure an intercalator that binds to DNA. To increase the antitumor activity of our cyclic compounds, we planned to synthesize derivatives that carried intercalative agents in their structures by linkage of different chromophores to the hydroxyl group of **15**.

Thus, the reaction of enediyne **15** and 9-fluorenylmethylchloroformate **17**, as shown in Scheme 5, using $KN(SiMe_3)_2$ gave carbonate **18** in 10% yield. It should be pointed out that in the mass spectrum of **18**, the maximum intensity peak corresponds to *m*/*e* 178 (100%), as in its precursor. All attempts to introduce naphthalimide rings into enediyne **15** using acid **19** under the esterification conditions reported by Mitsunobo[17] (Scheme 6), or the corresponding acid chloride, were unsuccessful.

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[15] In reference 6, we wrongly assigned the endocyclic double bond of isomer (*Z*)-**13** in position C_3-C_4. The performance of several experiments did not lead to the unambiguous determination of the structure. However, study of the cyclized derivative **15** allowed us the full assignment of the structure of aldehyde (*Z*)-**13**.

[16] Imamoto, T.; Sugiura, Y.; Takiyama, N. *Tetrahedron Lett.* **1984**, *25*, 4233.

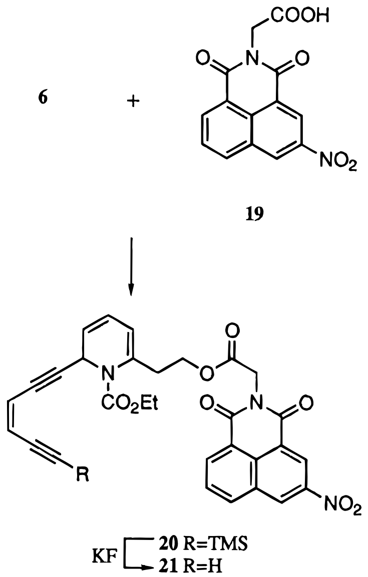
[17] Mitsunobu, O. *Synthesis* **1981**, 1.

Table 1. Cytotoxicity Values

compd	IC_{50} (mol/L)
9	1.00×10^{-3}
10	8.00×10^{-4}
11	4.00×10^{-5}
6	4.00×10^{-5}
14	1.00×10^{-5}
15	1.00×10^{-5}
18	1.00×10^{-5}
21	1.00×10^{-6}
A[a]	3.80×10^{-7}

[a] **A**: Amonafide.[8]

Scheme 6



The compounds designed and synthesized here were tested for in vitro cytotoxicity against the highly resistant HT-29 human colon carcinoma and compared to Amonafide,[8] a known naphthalimide antitumor agent. The IC_{50} cytotoxicity values are shown in Table 1.

After comparing the IC_{50} values of **6** with those of **14** and **15**, we concluded that cyclic strain, at least in this example, did not improve the antitumor activity. On the other hand, the instability of **14** and **15** suggested that it might also be interesting to prepare a derivative of **6** that contained the acyclic enediyne unit linked to an intercalator.

The first chromophore we intended to introduce was the fluorene ring. However, although several assays of the reaction of **6** with 9-fluorenylmethyl chloroformate **(17)** and different bases were carried out, none gave the expected compound. Better results were obtained with the naphthalimide chromophore. As shown in Scheme 6, the synthesis of ester **21** was accomplished in two steps.

The first step involved a Mitsunobo esterification (triphenylphosphine and diethyl azodicarboxylate in THF) of alcohol **6** and 3-nitro-1,8-naphthalimidoylacetic acid **19** to give ester **20** in 64% yield. Deprotection of the terminal alkyne with KF in DMF/H_2O led to **21** in 88% yield. Both compounds were characterized on the basis of their spectroscopic data.

We can conclude that the cyclic enediynes **14** and **15** are 100 times less active than Amonafide. In addition, the introduction of an intercalator in the structure of **15** did not improve the activity with reference to the precursor. However, it should be pointed out that the activity of the acyclic enediyne **6** is increased by the introduction of an intercalator, such as a naphthalimide ring.

Conclusions

In summary, these pyridinic derivatives did not show the expected cytotoxic activity, mainly due to their instability. However, the acyclic derivative shows enhanced activity when it possesses an intercalator, such as **21**, as carrier. Further studies to improve the antitumor activity of these compounds are underway.

Experimental Section

General. All reactions were carried out under a nitrogen atmosphere. Analytical TLC was carried out on E. Merck precoated HPTLC silica gel plates (60 F_{254}) with detection by UV. Column chromatography was performed using E. Merck 230-400 mesh silica gel. Melting points are uncorrected. Band positions in infrared spectra are indicated in wavenumbers. ^1H-NMR and ^{13}C-NMR spectra were recorded using $CDCl_3$ or acetone-d_6 as solvent. In both ^1H-NMR and ^{13}C-NMR, chemical shifts are reported in δ units downfield from TMS.

2-[2-(*tert*-Butyldimethylsilyloxy)ethyl]pyridine (2). To a stirred solution of 2-(2-hydroxyethyl)pyridine (**1**) (10 g, 81.2 mmol) in DMF (10 mL) was added imidazole (13.5 g, 198 mmol) and *tert*-butyldimethylsilyl chloride (14.5 g, 95 mmol). The solution was allowed to stir at rt for 24 h and then diluted with EtOAc (100 mL). The aqueous layer was extracted three times with 100 mL portions of EtOAc. The organic phase was dried with $MgSO_4$ and concentrated via rotary evaporation. The resulting oil was distilled to yield 18.2 g (95%) of **2** as a colorless oil. IR (film) 1255, 830, 770. ^1H-NMR ($CDCl_3$, 200 MHz) δ 0.08 (s, 6H), 0.80 (s, 9H), 2.97 (t, 2H, $J = 6.5$ Hz), 3.96 (t, 2H, $J = 6.5$ Hz), 7.11 (td, 1H, $J = 7.7$, 1.1 Hz), 7.19 (d, 1H, $J = 7.7$ Hz), 7.57 (td, 1H, $J = 7.7$, 1.8 Hz), 8.51 (dd, 1H, $J = 4.1$, 0.8 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ −3.7, 19.7, 27.1, 43.2, 64.3, 122.7, 125.5, 137.4, 150.6, 161.0. MS m/e (%): 237 (M^+, 5), 222 (47), 180 (95), 150 (31), 106 (95), 73 (100).

6-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-1-(ethoxycarbonyl)-2-[(trimethylsilyl)ethynyl]-1,2-dihydropyridine (3). To a stirred solution of (trimethylsilyl)acetylene (8.4 mL, 59 mmol) in THF (10 mL) at 0 °C were added a 1 M solution of ethylmagnesium bromide (60 mL, 59 mmol) via syringe, a solution of **2** (8.9 g, 37.5 mmol) in THF (17 mL), and a solution of ethyl chloroformate (5 mL, 52 mmol) in THF (17 mL). The reaction mixture was allowed to reach rt. After the solution was stirred for 20 h, the reaction was quenched with saturated aqueous NH_4Cl (200 mL). The aqueous layer was separated and extracted with EtOAc (3 × 200 mL). The organic phase was dried with $MgSO_4$ and concentrated. Purification by flash column chromatography gave 6.7 g (44%) of **3** as an orange oil. IR (film) 2140, 1700, 1240, 830, 750. ^1H-NMR ($CDCl_3$, 200 MHz) δ 0.06 (s, 6H), 0.20 (s, 9H), 0.89 (s, 9H), 1.31 (t, 3H, $J = 7.1$ Hz), 2.48−2.60 (m, 1H), 2.93−3.12 (m, 1H), 3.63−3.85 (m, 2H), 4.15−4.30 (m, 2H), 5.62−5.97 (m, 4H).

1-(Ethoxycarbonyl)-6-(2-hydroxyethyl)-2-[(trimethylsilyl)ethynyl]-1,2-dihydropyridine (4). To a stirred solution of **3** (6.6 g, 16 mmol) in acetonitrile (30 mL) at rt was added HF (40% in water) (1.4 mL, 32 mmol). The reaction was stirred for 8 h. The solvent was removed on a rotary evaporator, and the resulting slurry was diluted with saturated aqueous $NaHCO_3$ (100 mL) and extracted with EtOAc (3 × 100 mL). The organic phase was dried with $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (toluene/EtOAc, 9.5:0.5) yielded 2.7 g (60%) of **4** as a brown oil. IR (film) 3400, 2160, 1700, 1250, 850, 760. ^1H-NMR ($CDCl_3$, 200 MHz) δ 0.14 (s, 9H), 1.29 (t, 3H, $J = 7.1$ Hz), 1.63 (s, 1H), 2.36−2.51 (m, 1H), 3.10−3.30 (m, 1H), 3.64−3.78 (m, 2H), 4.15−4.25 (m, 2H), 5.70−6.02 (m, 4H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ −0.4, 14.3, 38.2, 44.3, 60.2, 62.4, 87.9, 102.6, 115.5, 121.5, 123.2, 135.3, 154.0.

2*H*,4*H*-4-Ethynyl-8,9-dihydropyrido[1,2-*c*][1,3]oxazin-2-one (9). To a stirred solution of **4** (4.3 g, 14 mmol) in MeOH (35 mL) was added anhydrous K_2CO_3 (177 mg, 1.2 mmol). The reaction mixture was stirred for 1 h at rt. The solvent was removed in vacuo, and the resulting slurry was diluted with EtOAc (50 mL) and washed with water. The organic phase was dried with $MgSO_4$, concentrated, and chromatographed (toluene/EtOAc, 7:3) to yield 1.4 g (58%) of **9** as a white solid. Mp 76−77 °C. IR (KBr) 3280, 2100, 1700. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 2.38 (d, 1H, J = 2.1 Hz, ≡CH), 2.51 (dt, 1H, J = 15.3, 3.0 Hz, H_8), 2.85 (dddd, 1H, J = 15.3, 11.6, 5.3, 1.9 Hz, $H_{8'}$), 4.26 (ddd, 1H, J = 11.6, 10.7, 3.0 Hz, H_9), 4.38 (ddd, 1H, J = 10.7, 5.3, 3.0 Hz, $H_{9'}$), 5.16 (dt, 1H, J = 5.7, 1.9, 1.1 Hz, H_7), 5.61 (ddd, 1H, J = 8.8, 6.0, 1.1 Hz, H_5), 5.65 (dd, 1H, J = 6.0, 2.1 Hz, H_4), 5.94 (dd, 1H, J = 8.8, 5.7 Hz, H_6). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 27.6 (CH_2), 43.9 (CH), 65.1 (CH_2O), 81.0 (C≡*C*H), 102.5 (*C*≡CH), 118.0, 122.1, 123.2, 132.6 (C═C), 149.2 (CO). MS *m/e* (%): 175 (M^+, 26), 130 (100), 116 (5), 103 (15), 77 (18), 65 (6). Anal. Calcd for $C_{10}H_9NO_2$: C, 68.57; H, 5.14; N, 8.00. Found: C, 68.39; H, 5.20; N, 7.70.

6-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-2-ethynyl-1-(ethoxycarbonyl)-1,2-dihydropyridine (10). To a stirred solution of **2** (10 g, 42 mmol) in THF (30 mL) at −40 °C was added a solution of ethyl chloroformate (4.6 mL, 49 mmol) in THF (30 mL) dropwise. After complete addition and 1 h stirring, a 0.5 M solution of ethynylmagnesium bromide in THF (133 mL, 66.5 mmol) was added via syringe at 0 °C. The mixture was stirred for 3 h at 0 °C, and 8 h at rt. The reaction was quenched with saturated aqueous NH_4Cl (200 mL). The aqueous layer was separated and extracted with EtOAc (3 × 100 mL). The organic phase was dried with $MgSO_4$ and concentrated. The product was purified by flash column chromatography (toluene/EtOAc, 9.5:0.5) to yield 8.7 g (62%) of **10** as a yellow oil. IR (film) 3300, 2100, 1700, 1250, 830, 770. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.05 (s, 6H), 0.89 (s, 9H), 1.32 (t, 3H, J = 7.1 Hz), 2.23 (d, 1H, J = 7.2 Hz), 2.46−2.61 (m, 1H), 2.97−3.11 (m, 1H), 3.76 (t, 2H, J = 7.2 Hz), 4.17−4.29 (m, 2H), 5.64−5.74 (m, 3H), 5.96−6.02 (m, 1H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ −3.7, 15.9, 19.7, 27.2, 39.0, 45.3, 63.4, 64.0, 72.3, 82.3, 121.9, 129.7, 130.5, 139.3, 152.2. MS *m/e* (%): 335 (M^+, 36), 320 (6), 278 (95), 262 (24), 232 (13), 204 (41), 180 (28), 158 (52), 130 (95), 103 (95), 73 (100).

6-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-1-(ethoxycarbonyl)-2-[6-(trimethylsilyl)-3(*Z*)-hexene-1,5-diynyl]-1,2-dihydropyridine (11). To a stirred solution of **10** (10 g, 30 mmol) in toluene (25 mL) were added (*Z*)-(4-chloro-3-buten-1-ynyl)trimethylsilane (7.3 g, 45.6 mmol) in toluene (10 mL), $Pd(PPh_3)_4$ (1.7 g, 1.5 mmol), CuI (1.1 g, 5.7 mmol), and *n*-butylamine (4.6 mL, 18 mmol). The mixture was stirred for 6 h. The slurry was filtered, and the solid was washed several times with EtOAc. The filtrate was washed with water (3 × 100 mL). The organic layer was dried with $MgSO_4$, concentrated, and purified by flash column chromatography (toluene/hexane, 9:1) to yield 6.3 g (46%) of **11** as a brown oil. IR (film) 2120, 1700, 1250, 840, 760. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.02 (s, 6H), 0.19 (s, 9H), 0.86 (s, 9H), 1.33 (t, 3H, J = 7.1 Hz), 2.43−2.58 (m, 1H), 2.94−3.08 (m, 1H), 3.68−3.81 (m, 2H), 4.12−4.45 (m, 2H), 5.61−5.99 (m, 6H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ −5.3, −0.2, 14.4, 18.3, 25.8, 37.5, 44.6, 58.7, 62.3, 79.8, 94.1, 101.7, 102.8, 113.4, 120.0, 120.6, 124.2, 126.7, 135.8, 153.7. MS *m/e* (%): 457 (M^+, 35), 400 (21), 312 (16), 253 (18), 180 (17), 133 (18), 103 (15), 73 (100).

1-(Ethoxycarbonyl)-6-(2-hydroxyethyl)-2-[6-(trimethylsilyl)-3(*Z*)-hexene-1,5-diynyl]-1,2-dihydropyridine (6). To a stirred solution of **11** (4.5 g, 9.8 mmol) in acetonitrile (56 mL) at −20 °C was added HF (40% in water) (0.6 mL, 13.8 mmol). The reaction was stirred for 8 h. The solvent was removed on a rotary evaporator, and the resulting slurry was diluted with saturated aqueous $NaHCO_3$ (100 mL) and extracted with EtOAc (3 × 100 mL). The organic phase was dried with $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (toluene/EtOAc, 7:3) yielded 2.8 g (85%) of **6** as a green oil. IR (film) 3450, 2120, 1700, 1250, 850, 760. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.21 (s, 9H), 1.30 (t, 3H, J = 7.1 Hz), 2.06 (s, 1H), 2.38−2.53 (m, 1H), 3.18−3.26 (m, 1H), 3.63−3.84 (m, 2H), 4.10−4.30 (m, 2H), 5.71−6.05 (m, 6H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ −0.3, 14.3, 37.7, 44.4, 60.3, 62.5, 79.9, 93.8, 101.4, 103.3, 114.9, 119.2, 120.7, 121.0, 123.3, 135.4, 153.5. MS *m/e* (%): 343 (M^+, 2), 312 (5), 282 (4), 252 (17), 224 (18), 180 (16), 73 (100).

6-(Formylmethyl)-1-(ethoxycarbonyl)-2-[6-(trimethylsilyl)-3(*Z*)-hexene-1,5-diynyl]-1,2-dihydropyridine (7). A 250 mL round bottom flask was charged with dry CH_2Cl_2 (42 mL) and cooled to −78 °C, and oxalyl chloride (1.9 mL, 22 mmol) was added. Distilled dry DMSO (3.4 mL, 42.5 mmol) in CH_2Cl_2 (44 mL) was then added dropwise, and the reaction mixture was allowed to stir for 15 min at −78 °C. Alcohol **6** (5.8 g, 17 mmol) dissolved in dry CH_2Cl_2 (30 mL) was slowly added. The reaction mixture was allowed to stir for 6 h. Et_3N was then added (11.6 mL, 82.2 mmol), and the flask was removed from the cold bath. The mixture was allowed to react at rt and was stirred 30 min before addition of water (100 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic extracts were washed with HCl (10%) (50 mL), H_2O (50 mL), Na_2CO_3 (10%) (50 mL), and H_2O (50 mL). The organic layer was dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The product was purified by flash column chromatography (hexane/EtOAc, 7:3) to yield 2.6 g (45%) of **7** as a brown oil. IR (film) 2150, 1700, 1250, 850, 760. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.20 (s, 9H), 1.28 (t, 3H, J = 7.1 Hz), 3.29−3.58 (m, 2H), 4.15−4.27 (m, 2H), 5.60−6.08 (m, 6H), 9.67 (dd, 1H, J = 2.6, 1.1 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ −0.2, 14.3, 44.6, 48.3, 62.8, 80.3, 93.4, 101.5, 103.1, 115.4, 118.9, 119.7, 120.2, 122.8, 130.8, 153.4, 198.4.

(*E*)-1-(Ethoxycarbonyl)-6-(formylmethylidene)-2-[(*Z*)-hexene-1,5-diynyl]-1,2,3,6-tetrahydropyridine (12) and (*Z*)-1-(Ethoxycarbonyl)-2-(formylmethylidene)-6-[(*Z*)-hexene-1,5-diynyl]-1,2,3,6-tetrahydropyridine (13). To a solution of **7** (2.2 g, 6.4 mmol) in a mixture of DMF (38 mL) and H_2O (1.4 mL) was added KF (0.55 g, 9.5 mmol). The reaction mixture was stirred at rt. After 3 h of stirring, water was added (50 mL), and the mixture was extracted with CH_2Cl_2 (5 × 50 mL). The organic phase was washed with water and then dried with $MgSO_4$. Evaporation of solvent and flash chromatography (toluene/EtOAc, 9.5:0.5) yielded a 2:1 mixture of **12** (40%) and **13** (20%) as yellow oils. Data of **12**: IR (film) 3280, 2180, 1700. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.33 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.55 (dd, 1H, J = 18.5, <1 Hz, H_{3a}), 2.79 (m, 1H, H_{3e}), 3.25 (dd, 1H, J = 2.2, <1 Hz, H_{11}), 4.30 (m, 2H, CH_2-CH_3), 5.74 (dd, 1H, J = 6.2, 1.8 Hz, H_2), 5.78 (dd, 1H, J = 11.0, 2.2 Hz, H_{10}), 5.83 (dd, 1H, J = 11.0, <1 Hz, H_9), 6.28 (m, 1H, H_4), 6.40 (d, 1H, J = 7.4 Hz, H_7), 7.13 (dd, 1H, J = 10.2, 3.1 Hz, H_5), 10.13 (d, 1H, J = 7.4 Hz, H_8). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 14.3, 31.3, 44.7, 63.3, 79.9, 80.3, 85.0, 94.3, 118.6, 119.9, 120.5, 120.7, 131.6, 147.7, 153.1, 189.6. MS *m/e* (%): 269 (M^+, 6), 240 (43), 224 (6), 212 (24), 196 (76), 180 (30), 167 (100), 152 (75), 141 (53), 136 (49), 115 (53), 102 (25), 89 (27), 77 (33), 65 (39), 63 (40). Data of **13**: IR (film) 3280, 2090, 1700. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.33 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.48 (dd, 1H, J = 18.7, <1 Hz, H_{3a}), 2.81 (m, 1H, H_{3e}), 3.23 (d, 1H, J = 2.2 Hz, H_{11}), 4.15 (m, 2H, CH_2CH_3), 5.78 (dd, 1H, J = 11.3, 2.2 Hz, H_{10}), 5.79 (m, 2H, H_2, H_7), 5.82 (dd, 1H, J = 11.0 Hz, H_9), 6.16 (m, 1H, H_4), 6.24 (dd, 1H, J = 9.9, 2.8 Hz, H_5), 9.69 (d, 1H, J = 7.7 Hz, H_8). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 14.1, 38.8, 44.9, 63.4, 80.2, 85.1, 93.9, 119.5, 120.4, 122.6, 128.2, 131.8, 153.5, 190.8. MS *m/e* (%): 269 (M^+, 7), 240 (43), 224 (6), 212 (23), 196 (83), 181 (21), 167 (100), 152 (77), 141 (49), 136 (54), 115 (46), 102 (20), 89 (24), 77 (31), 65 (33), 63 (31).

(4*Z*,9*E*)-14-(Ethoxycarbonyl)-8-hydroxy-14-azabicyclo[8.3.1]tetradeca-4,9,11-triene-2,6-diyne (14). To a stirred solution of **12** (283 mg, 1.0 mmol) in THF (10 mL) at −78°C was added $LiNSiMe_3$ (210 mg, 1.3 mmol) and $CeCl_3$ (310 mg, 1.3 mmol). The solution was stirred for 30 min, and the reaction was quenched with water. The aqueous layer was extracted with EtOAc (3 × 30 mL). The organic phase was dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (toluene/EtOAc, 9:1) to yield 194 mg (51%) of **14** as a brown oil. IR (film) 3400, 1680. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.28 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.20−2.89 (m, 3H, CH_2, OH), 4.07−4.28 (m, 2H, CH_2CH_3), 5.44 (d, 1H, J = 1.8 Hz, H_1), 5.59 (d, 1H, J = 8.2 Hz, H_8), 5.71−5.89 (m, 4H,

H_4, H_5, H_9, H_{12}), 6.39 (dd, 1H, J = 10.2, 2.7 Hz, H_{11}). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 14.5, 31.8, 46.7, 60.7, 62.1, 83.6, 86.8, 95.4, 99.6, 120.8, 122.2, 122.8, 125.4, 128.2, 131.6, 153.1. MS *m/e* (%): 269 (M^+, 49), 240 (12), 221 (9), 196 (39), 178 (28), 167 (53), 111 (33), 83 (53), 57 (100).

(4*Z*,9*Z*)-14-(Ethoxycarbonyl)-8-hydroxy-14-azabicyclo[8.3.1]tetradeca-4,9,11-triene-2,6-diyne (15). To a stirred solution of **13** (380 mg, 1.4 mmol) in THF (10 mL) at −78°C were added $LiNSiMe_3$ (290 mg, 1.7 mmol) and $CeCl_3$ (420 mg, 1.7 mmol). The solution was stirred for 30 min, and the reaction was quenched with water. The aqueous layer was extracted with EtOAc (3 × 30 mL). The organic phase was dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (toluene/EtOAc, 9:1) to yield 130 mg (35%) of **15** as a brown oil. IR (film) 3400, 1680. ^{1}H-NMR (acetone-d_6, 500 MHz) δ 1.29 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.17−2.78 (m, 2H, CH_2), 2.79 (1H, s, OH), 4.10−4.30 (m, 2H, CH_2CH_3), 5.61−5.62 (m, 2H, H_1, H_8), 5.76−5.85 (m, 4H, H_4, H_5, H_9, H_{12}), 6.12 (dd, 1H, J = 10.1, 2.6 Hz, H_9). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 14.5, 28.7, 44.9, 60.4, 62.5, 83.0, 84.8, 97.5, 100.6, 121.5, 124.8, 127.0, 128.1, 128.9, 131.4, 153.0. MS *m/e* (%): 269 (M^+, 18), 251 (42), 240 (5), 222 (12), 206 (6), 196 (27), 178 (100), 167 (31), 152 (66).

(4*Z*,9*Z*)-14-(Ethoxycarbonyl)-8-[[(9-fluorenylmethoxy)carbonyl]oxy]-14-azabicyclo[8.3.1]tetradeca-4,9,11-triene-2,6-diyne (18). To a stirred solution of **15** (100 mg, 0.4 mmol) in THF (5 mL) at 0 °C were added $KN(SiMe_3)_2$ (90 mg, 0.5 mmol) and 9-fluorenylmethyl chloroformate **17** (100 mg, 0.4 mmol). The solution was allowed to reach rt and was stirred for 10 h. The reaction was quenched with water. The aqueous layer was extracted with EtOAc (3 × 20 mL). The organic phase was dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (hexane/EtOAc, 8:2) to yield 200 mg (10%) of **18** as a yellow oil. IR (CH_2Cl_2) 1750, 1700. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.25 (t, 3H, J = 7.1 Hz), 2.20−2.85 (m, 2H), 4.23−4.30 (m, 3H), 4.43 (d, 2H, J = 6.4 Hz), 5.74−5.92 (m, 5H), 6.12 (dd, 1H, J = 10.2, 2.5 Hz), 6.45 (d, 1H, J = 6.9 Hz), 7.31 (td, 2H, J = 7.4, 1.2 Hz), 7.41 (td, 2H, J = 7.4, 1.2 Hz), 7.62 (dd, 2H, J = 7.0, 1.8 Hz), 7.76 (d, 2H, J = 7.0 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 14.5, 28.7, 44.9, 46.6, 60.4, 62.9, 70.3, 85.1, 86.9, 97.8, 98.0, 120.0 (3), 124.7, 125.2 (2), 125.8 (3), 127.2 (3), 127.9 (3), 128.0, 141.3 (2), 152.8, 154.1. MS *m/e* (%): 491 (M^+, 0.2), 342 (0.3), 269 (3), 251 (4), 238 (2), 222 (1), 196 (15), 178 (100), 165 (37), 152 (37), 76 (16).

6-{2-[[[(1,3-Dioxo-5-nitrobenzo[*d,e*]isoquinolin-2-yl)methyl]carbonyl]oxy]ethyl}-1-(ethoxycarbonyl)-2-[6-(trimethylsilyl)-3(*Z*)-hexene-1,5-diynyl]-1,2-dihydropyridine (20). To a stirred solution of **6** (200 mg, 0.6 mmol) in THF (10 mL) were added 1,3-dioxo-5-nitrobenzo[*d,e*]isoquinolin-2-ylacetic acid (**19**) (300 mg, 1.2 mmol), triphenylphosphine (300 mg, 1.2 mmol), and diethyl azodicarboxylate (200 mg, 1.2 mmol). The reaction mixture was stirred for 2 h. The solvent was removed in vacuo and chromatographed (toluene/EtOAc, 9:1) to yield 230 mg (64%) of **20** as a yellow oil. IR (film) 2140, 1740, 1710, 1670, 1530, 1340, 1250, 850, 760. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.20 (s, 9H), 1.31 (t, 3H, J = 7.1 Hz), 2.62−2.69 (m, 1H), 3.22−3.35 (m, 1H), 4.18−4.41 (m, 4H), 4.93 (s, 2H), 5.64−5.97 (m, 6H), 7.95 (t, 1H, J = 7.8 Hz), 8.45 (dd, 1H, J = 8.2, 0.9 Hz), 8.77 (dd, 1H, J = 7.3, 1.1 Hz), 9.16 (d, 1H, J = 2.3 Hz), 9.30 (d, 1H, J = 2.3 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ −0.2 (3), 14.4, 33.3, 44.6, 60.4, 62.6, 64.0, 80.3, 93.8, 101.8, 102.9, 114.3, 120.1 (2), 121.2, 122.7, 123.1, 124.2, 124.5, 129.1, 129.3, 130.3, 131.0, 134.4, 134.7, 135.9, 146.3, 153.6, 162.0, 162.6, 167.5. MS *m/e* (%): 626 (M^+ + 1, 2), 478 (3), 326 (8), 255 (23), 180 (10), 133 (29), 73 (100).

6-{2-[[[(1,3-Dioxo-5-nitrobenzo[*d,e*]isoquinolin-2-yl)methyl]carbonyl]oxy]ethyl}-1-(ethoxycarbonyl)-2-[3(*Z*)-hexen-1,5-diynyl]-1,2-dihydropyridine (21). To a solution of **20** (200 mg, 0.32 mmol) in DMF (3 mL) and H_2O (0.1 mL) was added KF (30 mg, 0.5 mmol). The reaction mixture was stirred at rt. After 5 h of stirring, water was added (20 mL), and the mixture was extracted with CH_2Cl_2 (5 × 20 mL). The combined organic extracts were washed with water and then dried with $MgSO_4$. Evaporation of solvent and flash column chromatography (toluene/EtOAc, 9:1) yielded 150 mg (88%) of **21** as a yellow oil. IR (film) 3290, 2100, 1740, 1700, 1670, 1530, 1340. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.30 (t, 3H, J = 7.1 Hz), 2.58−2.73 (m, 1H), 3.22−3.29 (m, 1H), 3.35 (d, 1H, J = 2.2 Hz), 4.04−4.43 (m, 4H), 4.92 (s, 2H), 5.63−5.96 (m, 6H), 7.93 (t, 1H, J = 7.8 Hz), 8.45 (dd, 1H, J = 8.2, 0.9 Hz), 8.74 (dd, 1H, J = 7.3, 1.1 Hz), 9.14 (d, 1H, J = 2.3 Hz), 9.26 (d, 1H, J = 2.3 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 14.3, 33.2, 44.5, 60.3, 62.6, 64.1, 80.0, 80.4, 84.9, 93.8, 114.1, 118.8, 120.9 (2), 122.6, 123.1, 124.0, 124.4, 129.1, 129.3, 130.2, 130.9, 134.3, 134.6, 135.9, 146.2, 153.5, 162.0, 162.6, 167.5. MS *m/e* (%): 553 (M^+, 6), 480 (10), 255 (50), 180 (40), 133 (100), 73 (72).

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Supporting Information Available: ^{1}H NMR spectra of compounds **2**−**4**, **6**, **7**, **10**−**15**, **18**, **20**. and **21** and ^{1}H and ^{13}C NMR full assignment of peaks (17 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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