Novel Amination and 1,2-Amino,hydro-Elimination between 2,3-Diketopyrido[4,3,2-*de*]quinolines and Primary Amino Compounds

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A novel amination and 1,2-amino,hydro-elimination reaction occurs between 2,3-diketopyrido[4,3,2-*de*]quinoline (**1**, **2**) and amino compounds which include α-amino acids and peptides which contain a primary amino group. The amino group undergoes nucleophilic addition to the double bond between C3a and C4 in 2,3-diketopyrido[4,3,2-*de*]quinoline to form a 3a,4-dihydro-2,3-diketopyrido-[4,3,2-*de*]quinoline. This dihydro intermediate is immediately oxidized by ambient air to produce the more stable aromatic system, 4-(*N*-alkyl or aryl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**5**−**12**). In the cases of aliphatic amines bearing a *β*-proton in THF or chloroform, this 4-(*N*-alkyl)-2,3-diketopyrido[4,3,2-*de*]quinoline undergoes a 1,2-amino,hydro-elimination reaction to eliminate an alkene and produce the 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**, **14**). In the cases of α-amino acids in aqueous solution, the 4-(*N*-alkyl)-2,3-diketopyrido[4,3,2-*de*]quinoline undergoes an amino-transferring reaction, via a mechanism similar to the action of pyridoxal, to form the 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**) and the α-keto acid. 2,3-Diketopyrido[4,3,2-*de*]quinoline (**1**) can also react with the peptide which contains a primary amino group to form the 2,3-diketopyrido-[4,3,2-*de*]quinoline−peptide conjugate. This novel amination−elimination reaction may underlie the marked cytotoxic potency of the 2,3-diketopyrido[4,3,2-*de*]quinolines (**1** and **2**). As inorganic amino compounds, hydroxylamine and hydrazine can also undergo the nucleophilic addition to 2,3-diketopyrido[4,3,2-*de*]quinoline (**1**, **2**) to produce 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**, **14**) which includes a elimination reaction between C4 and α-nitrogen.

Introduction

The physical and chemical properties of the marine environment are quite different from those of the terrestrial environment.[1] During the last 30 years, organic chemists have intensively studied the chemistry of marine organisms and begun to investigate the molecular structure and the biomedical potential of the metabolites from marine sources.[1,2] With researchers developing an increasing interest in marine research, many marine products have been examined in anticipation of new chemistry or desirable physiological activity. For example, the pyrido[4,3,2-*de*]quinoline core structure has been found in many sponges and tunicates (Chart 1). The chemistry and pharmacological properties of these marine natural products have been the subject of much current interest.[2,3] Many of these marine products have generated interest both as challenging problems for structure elucidation[4−11] and synthesis,[12−17] as well as for their biological activities.[18−28]

Amphimedine was isolated from a pacific sponge (amphimedon sp.) as a cytotoxic compound in 1983 and was the first example of the pyridoacridine alkaloids

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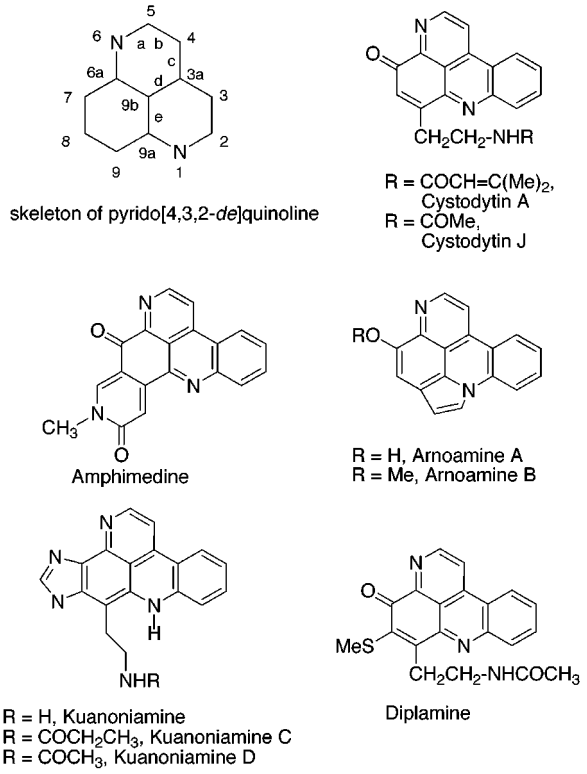
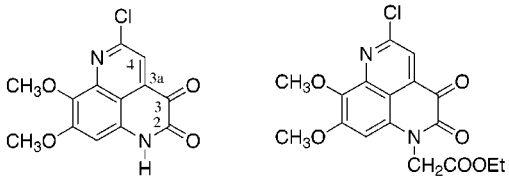
Chart 1. The Skeleton of Pyrido[4,3,2-*de*]quinoline and Structures of Amphimedine, Cystodytin, Aroamine, Kuanoniamine, Diplamine, and Aroamine



which contain a pyrido[4,3,2-*de*]quinoline core structure.[29] Cystodytin J and diplamine inhibit HCT replication with IC_{50} values less than 2 μM.[30] The pharmacological effects of kuanoniamine, kuanoniamine C, and kuanoniamine D were also studied.[31] The IC_{50} values of all these compounds against human cell lines HeLa and MONO-MAC-6 are less than 5.5 μM. Arnoamine A and B are proposed to be the first members of a new class of pentacyclic pyridoacridine alkaloids possessing a pyrrole ring fused to the pyridoacridine ring system.[32] Arnoamine A exhibits selective cytotoxicity against the MCF breast cancer cell line with a GI_{50} value of 0.3 μg/mL versus GI_{50}s of 2 and 4 μg/mL against A-549 and HT-29 cell lines, respectively. Arnoamine B exhibits GI_{50} of 5.0, 2.0, and 3.0 μg/mL against the MCF-7, A-549, and HT-29 cell lines, respectively. Initial assays showed differential toxicity between the DNA repair-deficient CHO cell line xrs-6 and the DNA repair-proficient CHO cell line BR1, suggesting a mechanism of action involving topoisomerase II inhibition. The new alkaloids provide useful lead structures for the development of new types of antitumor agents, a goal that would certainly be facilitated by the clarification of the mode of action of pyridoquinoline.

In previous papers, we have reported the synthesis, cytotoxic potency and anticancer activities of some pyrrolo-

Chart 2. Structures of Pyrido[4,3,2-*de*]quinoline 1 and 2



[4,3,2-*de*]quinolines[33−36] and pyrido[4,3,2-*de*]quinolines.[37−39] The biological evaluation data shows that pyrido[4,3,2-*de*]quinoline **1** and **2** (Chart 2) exhibit significant cytotoxic potency. Compound **2** exhibits a wide spectrum of anticancer activities against 22 cell lines in seven cancer panels with LC_{50} values less than 9 μM, especially in nonsmall cell lung cancer, melanoma cancer, renal cancer, prostate cancer, and breast cancer. Compounds **1** selectively affects the cell growth against nonsmall cell lung cancer HOP-92 cell line, breast cancer MDA-MB-435, and MDA-N cell lines with LC_{50} values less than 8 μM. The promising results of these compounds have aroused our interest in investigating the chemistry and the biochemistry of the novel 2,3-diketopyrido[4,3,2-*de*]-quinoline alkaloids. We herein report a novel amination and elimination reaction between 2,3-diketopyrido[4,3,2-*de*]quinolines and amino compounds which include α-amino acids and peptides and that may be related to their observed cytotoxic potency.

Results and Discussion

1. Amination and 1,2-Amino,hydro-Elimination between 2,3-Diketopyrido[4,3,2-*de*]quinoline and Primary Aliphatic Amines. In our previous studies, the C3 position in 2,3-diketopyrido[4,3,2-*de*]quinoline **1** and **2** was observed to be a highly active position toward nucleophilic addition. In the presence of organic bases, the C3 carbonyl group undergoes nucleophilic addition by acetone and acetophenone.[38,39] For example, compound **1** reacts with acetone in the present of triethylamine to form the *O*-hydro,*C*-acetonyl-addition product in high yield. In the present study, it was found that primary amino nucleophiles react with 2,3-diketopyrido[4,3,2-*de*]-quinoline **1** and **2** at the C4 position.

The *n*-propylamine can easily react with 2,3-diketopyrido[4,3,2-*de*]quinoline in THF to form a purple intermediate. This intermediate then forms a final product with red color. The HRMS results show that this red product has a composition which contains only one extra NH compared with the starting 2,3-diketopyrido[4,3,2-*de*]-quinoline. The ^{1}H NMR shows that there are two extra exchangeable protons contained in this product with different chemical shifts (9.85 and 6.33 ppm for the product from 2,3-diketopyrido[4,3,2-*de*]quinoline **2** and *n*-propylamine in $DCCl_3$). To determine where the nitro-

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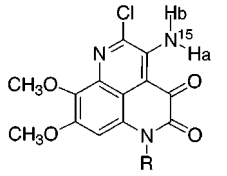
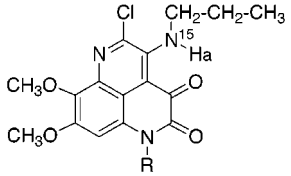


3: R = H
4: R = CH_2COOEt

Figure 1. Structures of ^{15}N-labeled 4-amino-2,3-diketopyrido-[4,3,2-*de*]quinoline **3** and **4**.



5: R = H
6: R = CH_2COOEt

Figure 2. Structures of ^{15}N-labeled 4-(N-propyl)amino-2,3-diketopyrido[4,3,2-*de*]quinoline **5** and **6**.

gen atom is located, an ^{15}N-labeled-*n*-propylamine was used for a similar reaction. The results of the isotope-labeling studies show that the red product contains one ^{15}N atom incorporated from the ^{15}N labeled *n*-propylamine. The ^1H NMR spectrum of the final product shows that both of the two exchangeable protons are attached to the ^{15}N atom with the same coupling constant but different chemical shifts, 9.88 ppm (d, 1 H, $^{15}N-Ha$, J $^{15}N-Ha = 92.5$ Hz) and 6.34 ppm (d, 1 H, $^{15}N-Hb$, J $^{15}N-Hb = 92.5$ Hz) for the red product from compound **2** in $DCCl_3$. NOE interactions reveal that the proton at 9.88 ppm (proton a) was affected by the proton at 6.34 ppm (proton b) at a level of 91%. The ^{15}N-hmqc (heteronuclear multiple quantum correlation) spectrum also confirms that both of these two protons are coupled with the ^{15}N atom. In addition, all of the NMR studies show that the red product lacks a aromatic proton compared with the starting 2,3-diketopyrido[4,3,2-*de*]quinoline. From these results, the structure of the red product was proposed as shown in structure **4** (Figure 1).

A hydrogen bond exists between the ^{15}NH and the 3-carbonyl group. This is presumably why these two protons on the nitrogen atom have different chemical shifts. The ^{15}N atom is derived from the ^{15}N labeled *n*-propylamine, and the *n*-propyl group has been removed from the ^{15}N atom. The overall result of the reaction between 2,3-diketopyrido[4,3,2-*de*]quinoline and *n*-propylamine is therefore that 2,3-diketopyrido [4,3,2-*de*]-quinoline extracted the amino group from *n*-propylamine.

To understand the mechanism of this novel reaction, an intermediate was isolated during the reaction of 2,3-diketopyrido[4,3,2-*de*]quinoline **2** with ^{15}N labeled *n*-propylamine. This intermediate is a purple product which is not very stable in THF at room temperature and is slowly transformed into the red product. Electrospray mass spectrum (ESMS) shows that the composition of this intermediate is only two protons less than the combination of starting 2,3-diketopyrido[4,3,2-*de*]quinoline and *n*-propylamine. The ^1H NMR studies of the ^{15}N labeled intermediate show that a proton and a *n*-propyl group are attached to the ^{15}N atom. This proton was coupled with ^{15}N atom with J $^{15}N-H = 92.5$ Hz. The ^{15}N-hmqc spectrum also confirms the coupling between ^{15}N and this proton. The results of ^{13}C NMR of this intermediate show that an aliphatic carbon (49.70 ppm, J ^{15}N, $^{13}C = 8.5$ Hz) and an aromatic carbon (146.15 ppm, J ^{15}N,$^{13}C = 15.8$ Hz) are directly linked to the ^{15}N atom. According to these results, the structure of this intermediate can therefore be proposed as shown in structure **6** (Figure 2).

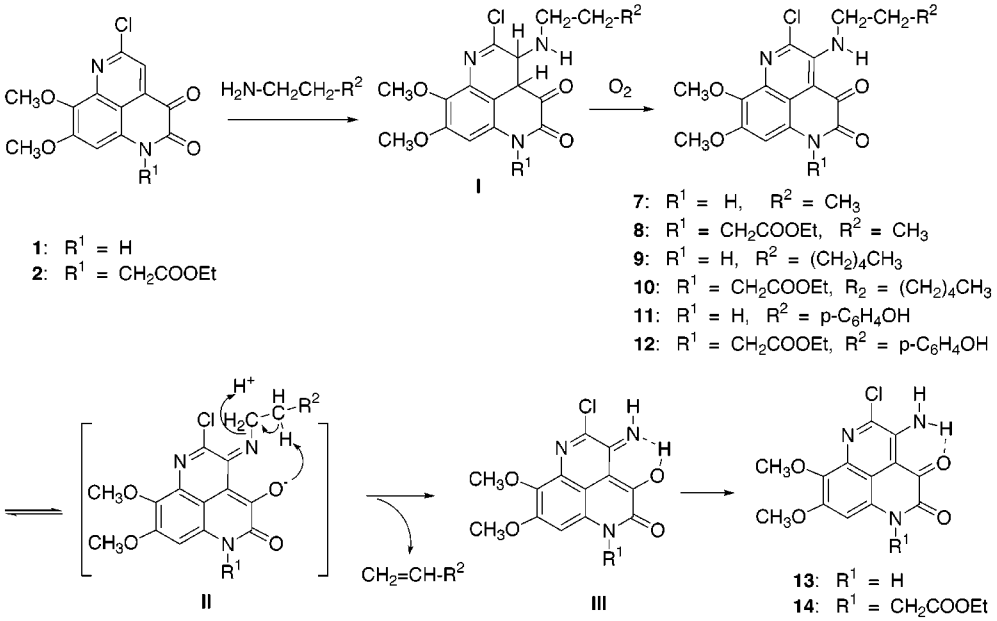
A hydrogen bond also exists between the $^{15}N-H$ and the 3-carbonyl group. It shifts this proton to lower field (δ $^{15}NH = 11.6$ ppm for compound **6** in $DCCl_3$). Evidently, this proton can be easily transferred from the N atom to the O atom at the C3 position to form a hydroxy group. In the presence of base this hydroxy group is deprotonated to produce a negative oxygen at the C3 position. The β-proton in the *n*-propyl group is located very close to this negative charged oxygen, and it is possible to transfer this β-proton onto the oxygen at the C3 position.

Both *n*-heptylamine and tyramine possess a β-proton and can also undergo a similar reaction in THF and generate the identical red product as was formed with *n*-propylamine. In case of tyramine a byproduct, *p*-hydroxystyrene, was identified by GC/MS technology as well as GC/IR technology. These results demonstrate that an 1,2-amino,hydro-elimination reaction is involved in the formation of *p*-hydroxystyrene. A novel amination−elimination mechanism was proposed for these reactions (Scheme 1).

The double bond between C3a and C4 is subject to nucleophilic addition by an amino group first to form a C3a,C4-dihydro intermediate (**I**). This dihydro intermediate is then air-oxidized to form the more stable aromatic system, 2,3-diketo-4-(*N*-alkyl)aminopyrido[4,3,2-*de*]quinoline (**7**−**12**). It was demonstrated that an oxidation reaction was involved in this amination reaction (see below). An 1,2-amino,hydro-elimination reaction was involved in this amination−elimination reaction which may be catalyzed by the 3-oxido ion. After the elimination reaction, a 2-keto-3-hydroxy-4-iminopyrido[4,3,2-*de*]quinoline (**III**) is formed and transformed immediately into the final product 4-amino-2,3-diketopyrido[4,3,2-*de*]-quinoline (**13**, **14**). The results of ^{15}N-labeling experiments (compound **3** and **4**) show the evidence favoring tautomer **13** and **14** over tautomer **III**.

2. Amination of 2,3-Diketopyrido[4,3,2-*de*]quinoline with Aromatic Amines. Aromatic amines such as aniline and 3,4-dimethoxyaniline can also react with 2,3-diketopyrido[4,3,2-*de*]quinoline to form a more stable purple product than that of aliphatic amines. For the purple product from 3,4-dimethoxyaniline and 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**2**), the HRMS shows that the composition is only two protons less than the combination of compound **2** and 3,4-dimethoxyaniline. ^1H NMR spectrum shows a exchangeable proton (12.27 ppm, NH) is contained in this product which indicates that this proton is involved in a hydrogen bond. To identify its structure, an X-ray diffraction analysis was carried out on a Bruker P4/RA/SMART 1000 CCD diffractometer. The result of the X-ray diffraction analysis shows that the 3,4-dimethoxyaniline group is covalently bonded to C4 in the 2,3-diketopyrido[4,3,2-*de*]-quinoline moiety (see Figure 3). A hydrogen bond exists between the N−H and the 3-carbonyl group as was previously deduced from NMR evidence. The plane of the benzene ring in 3,4-dimethoxyaniline group is rotated out off the pyrido[4,3,2-*de*]quinoline plane with a torsional

Scheme 1. Reaction of 2,3-Diketopyrido[4,3,2-*de*]quinolines with Aliphatic Amines



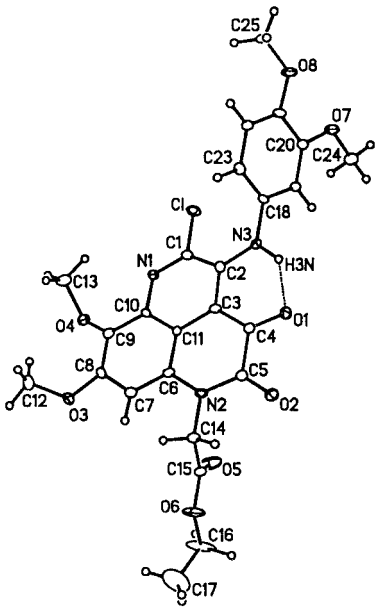
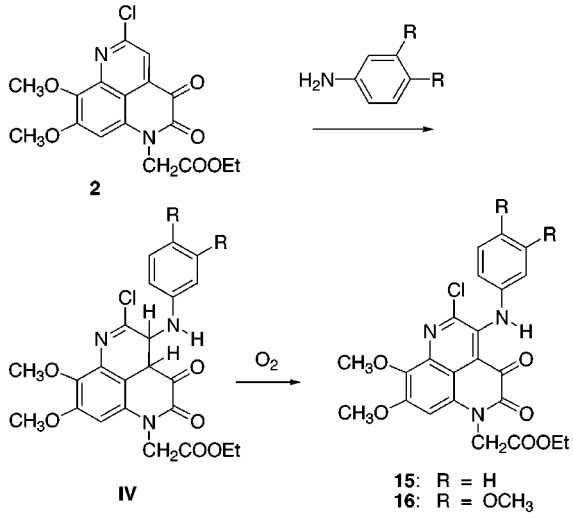


Figure 3. Perspective view of 4-(*N*-3,4-dimethoxyphenyl)-amino-2,3-diketopyrido[4,3,2-*de*]quinoline **16** showing the atom labeling scheme. Non-hydrogen atoms are represented by Gaussian ellipsoids at the 20% probability level.

Scheme 2. Reaction of 2,3-Diketopyrido[4,3,2-*de*]quinolines with Aromatic Amines

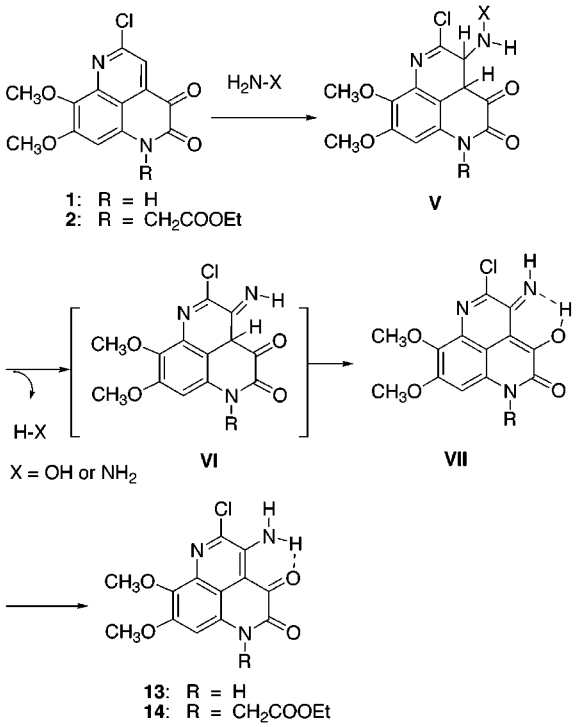


angles of 58.3° (angle-C2N3C18C23 = 58.3°, angle-C2N3C18C19 = −123.9°). The hydrogen bond stabilizes the carbonyl group at C3 position and inhibits the reaction of amino compound with this carbonyl group to form the Schiff base.

We propose a mechanism in which the nucleophilic addition by amino group takes place at the position-4 first to form the dihydro intermediate. This intermediate is then air-oxidized to form a more stable aromatic system (Scheme 2). The ratio of the oxygen involved in this reaction was determined as 1.0 mol of 2,3-diketopyrido-[4,3,2-*de*]quinoline to 0.5 mol of molecular oxygen in chloroform (see compound **15** in the Experimental Section). Hydrogen peroxide may be formed during the oxidation. This hydrogen peroxide can be used as an oxidant to oxidize the dihydro intermediate to form compound **15** and water.

3. Amination of 2,3-Diketopyrido[4,3,2-*de*]quinoline with NH_2OH and NH_2NH_2. Both hydroxylamine and hydrazine react with 2,3-diketopyrido[4,3,2-*de*]-quinoline **2** to produce a red product. This red product was identified by mp, ^1H NMR, and HRMS. All of these analytical results demonstrated that this red compound is 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline **14**, which is the same as that produced from the reaction of 2,3-diketopyrido[4,3,2-*de*]quinoline **2** with *n*-propylamine. Thus there is no doubt that the nitrogen atom at the 4 position in the red product originates from hydroxylamine or hydrazine. During the reaction, the OH group or the NH_2 group is eliminated to afford the red compound **14**. Compound **1** reacts with hydroxylamine or hydrazine in the manner similar to afford compound **13**. The amino group in hydroxylamine or hydrazine first undergoes a nucleophilic addition to the double bond between C3a and C4 to form a 3a,4-dihydro intermediate (**V**). This inter-

Scheme 3. Reaction of 2,3-Diketopyrido[4,3,2-*de*]quinolines with Hydroxylamine and Hydrazine

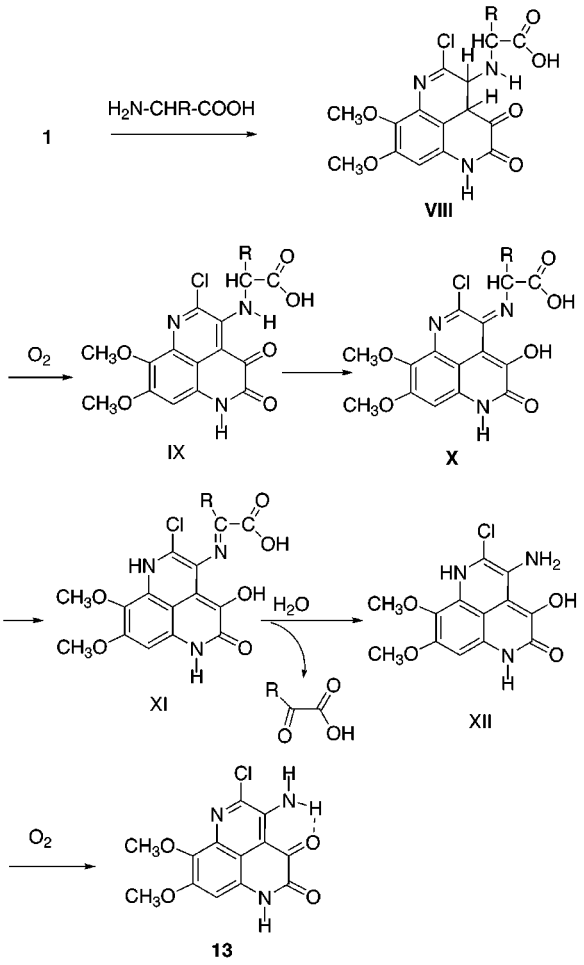


mediate then undergoes an elimination reaction between C4 and the amino group to form an imino group at the 4-position (**VI**). The proton at the C3a is then transferred to the oxygen at 3-position and finally transferred to the nitrogen at 4-position to afford the red product, 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**, **14**) (Scheme 3).

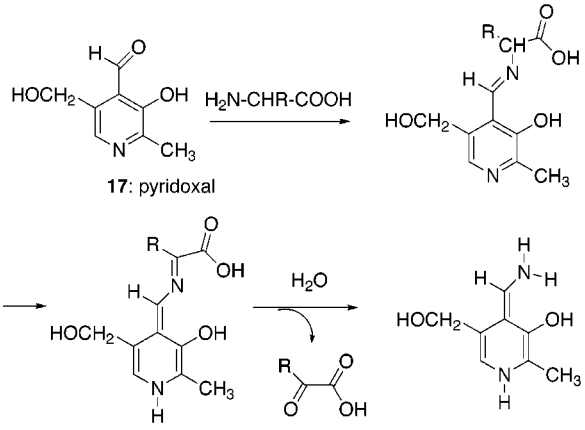
4. Amination and Elimination between 2,3-Diketopyrido[4,3,2-*de*]quinoline and α-Amino Acids. Glycine can react with 2,3-diketopyrido[4,3,2-*de*]quinoline (**1**) in aqueous solution to form the same product, 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**), as was formed with *n*-propylamine. Because glycine lacks a *β*-proton, the mechanism cannot follow that elucidated with *n*-propylamine, but resembles that with pyridoxal. Alanine, phenylanaline, and tryptophan can also react in a similar manner with 2,3-diketopyrido[4,3,2-*de*]quinoline (**1**) to produce 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**). The α-amino group undergoes nucleophilic addition to the double bond between C3a and C4 to form a dihydro intermediate (**VIII**) which is then oxidized to 4-(*N*-carboxymethyl)amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**IX**) (Scheme 4). In analogy with pyridoxal (**17**) (Scheme 5), in the presence of base, 4-(*N*-carboxymethyl)-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**IX**) can be transformed into 3-hydroxy-6-hydropyrido[4,3,2-*de*]quinoline (**XI**) which contains a carboxymethyleneamino group at the 4-position. The N=C double bond formed in this intermediate is then hydrolyzed by water to form an amino group at 4-position affording a product (**XII**) which is the reduced form of the final product of 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**).

5. Amination of 2,3-Diketopyrido[4,3,2-*de*]quinoline with Peptides. 2,3-Diketopyrido[4,3,2-*de*]quinoline **1** can react with peptides which contain an α-amino group or lysine residue to form 2,3-diketopyrido[4,3,2-*de*]quinoline−peptide conjugates. Substance P (**18**) is composed of 11 amino acids and contains an α-amino

Scheme 4. Reaction of 2,3-Diketopyrido[4,3,2-*de*]quinoline 1 with α-amino Acids

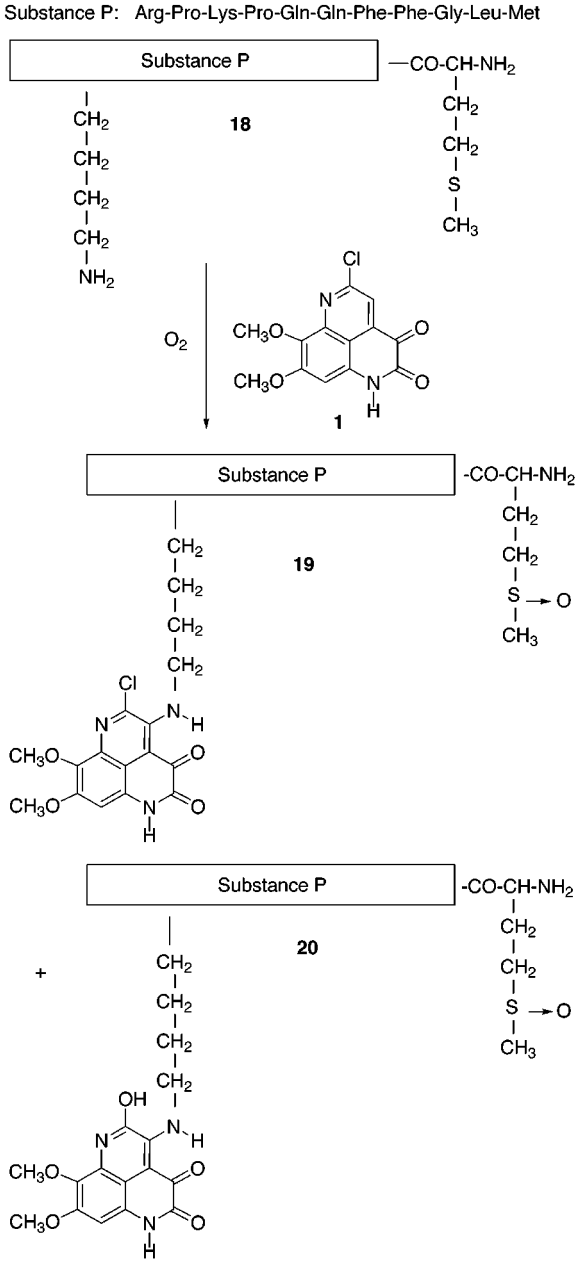


Scheme 5. Reaction of Pyridoxal with α-Amino Acids



group in methionine and an *ω*-amino group in lysine. After reaction with 2,3-diketopyrido[4,3,2-*de*]quinoline **1**, the 1:1 conjugate (**19**) and its hydrolysis product (**20**) were produced as the major products (Scheme 6). The 1:2 conjugate was also determined in the reaction solution by using the MALDI MS analysis technology. It is demonstrated that both of the amino groups in lysine and in methionine can react with 2,3-diketopyrido[4,3,2-*de*]-quinoline to form the conjugates. The CH_3S group was oxidized during this amination reaction. This may be caused by hydrogen peroxide which was produced by the

Scheme 6. Reaction of 2,3-Diketopyrido[4,3,2-*de*]quinoline 1 with Substance P



air-oxidation of the 3a,4-dihydro intermediate and will be examined further in a separate study.

Conclusions

The double bond between C3a and C4 in 2,3-diketopyrido[4,3,2-*de*]quinoline (**1** or **2**) is highly active site for nucleophilic addition. A novel amination−elimination reaction occurs between 2,3-diketopyrido[4,3,2-*de*]quinoline (**1**, **2**) and amino compounds which include α-amino acids and peptides. The amino group undergoes nucleophilic addition to the double bond between C3a and C4 to form a 3a,4-dihydro-2,3-diketopyrido[4,3,2-*de*]quinoline. This dihydro intermediate is immediately oxidized by ambient air to produce the more stable aromatic system, 4-(*N*-alkyl or aryl)-2,3-diketopyrido[4,3,2-*de*]-quinoline. In the cases of aliphatic amines bearing a *β*-proton in THF or chloroform, this 4-(*N*-alkyl)-2,3-diketopyrido[4,3,2-*de*]quinoline undergoes a 1,2-amino,-hydro-elimination reaction to eliminate an alkene and produce the 4-amino-2,3-diketopyrido[4,3,2-*de*]quinolines. In the cases of α-amino acids and compound **1** in aqueous solution, the 4-(*N*-alkyl)-2,3-diketopyrido[4,3,2-*de*]quinoline undergoes another deamination reaction, via a mechanism similar to the action of pyridoxal, to form the 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline **13** and the α-keto acid. 2,3-Diketopyrido[4,3,2-*de*]quinoline (**1**) can also react with peptides which contain primary amino groups to form the 2,3-diketopyrido[4,3,2-*de*]quinoline−peptide conjugates. This novel amination−elimination reaction may underlie the marked cytotoxic potency of the 2,3-diketopyrido[4,3,2-*de*]quinolines (**1** and **2**). As inorganic amino compounds, hydroxylamine and hydrazine can also undergo the nucleophilic addition to 2,3-diketopyrido[4,3,2-*de*]quinoline (**1**, **2**) to produce 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**, **14**), which includes an elimination reaction between C4 and α-nitrogen. The amination reaction between 2,3-diketopyrido[4,3,2-*de*]-quinoline and nucleotides as well as nucleic acids are ongoing.

Experimental Section

The hmqc ^{15}N spectra were recorded on the Varian Unity 500 MHz spectrometer. High-resolution mass spectra (HRMS) were recorded on a modified KRATOS MS-50 mass spectrometer equipped with a VG11−250J data system. Electrospray mass spectra were recorded on a Micromass ZabSpec spectrometer. MALDI MS analysis was performed on a Model G2025A time-of-flight system (Hewlett-Packard, Reno, NV). The X-ray diffraction analysis was carried out on a Bruker P4/RA/SMART 1000 CCD diffractometer. The programs were used in this analysis are DIRDIF-96 program system (Crystallography Laboratory, University of Nijmegen, The Netherlands) and SHELXL-93 program for crystal structure determination (University of Göttingen, Germany). Preparative separations were performed by flash chromatography on silica gel (Merck, 70−230 or 230−400 mesh). Substance P was purchased from Sigma and stored under −20 °C. Tetrahydrofuran was dried by distillation from sodium benzophenone ketyl. Triethylamine was dried over molecular sieves (3 Å) before use. All other solvents were used as received and were reagent grade where available.

1*H*-2,3-Diketo-4-amino(^{15}N)-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (3). A solution of 29 mg (0.10 mmol) of 1*H*-2,3-diketopyrido[4,3,2-*de*]quinoline (**1**) in 20 mL of THF was mixed with 100 mg of ^{15}N-labeled-*n*-propylamine hydrochloride ($CH_3CH_2CH_2{}^{15}N^+H_3Cl^-$) and 10 drops of triethylamine. The mixture was stirred at room temperature for 24 h. The resulting solution was then subjected to column chromatography (5:1 of ethyl acetate to hexane) to give 25 mg of the red product, 1*H*-2,3-diketo-4-amino(^{15}N)-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (**3**). Yield 83%, mp > 300 °C. HRMS: calcd for $C_{13}H_{10}{}^{14}N_2{}^{15}N_1O_4Cl$ 308.0360, found 308.0365 (M^+, 100%); ^1H NMR (DMSO-d_6): δ 11.92 (s, 1 H), 9.57 (d, 1 H, $^{15}N-Ha$, J $^{15}N-Ha$ = 92 Hz), 8.26 (d, 1 H, $^{15}N-Hb$, J $^{15}N-Hb$ = 92 Hz), 7.20 (s, 1 H), 3.90 (s, 3 H), 3.88 (s, 3 H). Anal. Calcd for $C_{13}H_{10}{}^{14}N_2{}^{15}N_1O_4Cl$: C, 50.58; H, 3.27; N, 13.93; Cl, 11.50. Found C, 50.26; H, 3.42; N, 13.67; Cl, 11.28.

1-(Ethoxycarbonylmethyl)-2,3-diketo-4-amino(^{15}N)-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (4). A solution of 38 mg (0.10 mmol) of 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**2**) in 20 mL of THF was mixed with 100 mg of ^{15}N labeled *n*-propylamine hydrochloride ($CH_3CH_2CH_2{}^{15}N^+H_3Cl^-$) and 10 drops of triethylamine. The mixture was stirred at room temperature for 24 h. The resulting solution was then subjected to column chromatography (1:1 of ethyl acetate to hexane then 4:1 of ethyl acetate to hexane) to give 35 mg of the red product, 1-(ethoxycarbonylmethyl)-2,3-diketo-4-amino(^{15}N)-5-chloro-7,8-dimethoxypy-

rido[4,3,2-*de*]quinoline (**4**). Yield 88%, mp 265−7 °C, HRMS: calcd for $C_{17}H_{16}{}^{14}N_2{}^{15}N_1O_6Cl$ 394.0698, found 394.0698 (M^+, 100%); ^{1}H NMR ($CDCl_3$): δ 9.88 (d, 1 H, ^{15}N−Ha, J ^{15}N−Ha = 92.5 Hz), 6.89 (s, 1 H), 6.34 (d, 1 H, ^{15}N−Hb, J ^{15}N−Hb = 92.5 Hz), 5.10 (s, 2 H), 4.26 (q, 2 H, J = 7.2 Hz), 4.13 (s, 3 H), 3.99 (s, 3 H), 1.28 (t, 3 H, J = 7.2 Hz). Anal. Calcd for $C_{17}H_{16}{}^{14}N_2{}^{15}N_1O_6Cl$: C, 51.72; H, 4.09; N, 10.89; Cl, 8.99. Found C, 51.48; H, 3.86; N, 10.96; Cl, 9.35.

1*H*-2,3-Diketo-4-(^{15}N-propyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (5). A solution of 29 mg (0.10 mmol) of 1*H*-2,3-diketopyrido[4,3,2-*de*]quinoline (**1**) in 20 mL of THF was mixed with 100 mg of ^{15}N labeled *n*-propylamine hydrochloride ($CH_3CH_2CH_2{}^{15}N^+H_3Cl^-$) and 10 drops of triethylamine. The mixture was stirred at room temperature for 3 h. The purple intermediate formed during the reaction was isolated immediately by flash chromatography (4:1 of ethyl acetate to hexane and then ethyl acetate) to give 20 mg of the purple 1*H*-2,3-diketo-4-(^{15}N-propyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (**5**). Yield 57%. ESMS: calcd for $C_{16}H_{16}{}^{14}N_2{}^{15}N_1O_4Cl$ 350.1, found 351.1 (MH^+, 100%); ^{1}H NMR (DMSO-d_6): δ 11.95 (s, 1 H), 10.85 (d, 1 H, ^{15}N−Ha, J ^{15}N−Ha = 92 Hz), 7.23 (s, 1 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.84 (m, 2 H, $^{15}NCH_2$), 1.72 (m, 2 H), 1.02 (t, 3 H, J = 7.2 Hz).

1-(Ethoxycarbonylmethyl)-2,3-diketo-4-(^{15}N-propyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (6). A solution of 38 mg (0.10 mmol) of 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**2**) in 20 mL of THF was mixed with 100 mg of ^{15}N labeled *n*-propylamine hydrochloride ($CH_3CH_2CH_2{}^{15}N^+H_3Cl^-$) and 10 drops of triethylamine. The mixture was stirred at room temperature for 0.5 h. The purple intermediate formed during the reaction was isolated immediately by flash chromatography (1:1 of ethyl acetate to hexane and then 4:1 of ethyl acetate to hexane) to give 32 mg of the purple 1-(ethoxycarbonylmethyl)-2,3-diketo-4-(^{15}N-propyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]-quinoline (**6**). Yield 73%. ESMS: calcd for $C_{20}H_{22}{}^{14}N_2{}^{15}N_1O_6Cl$ 436.1, found 437.1 (MH^+, 100%); ^{1}H NMR ($CDCl_3$): δ 11.62 (d, 1 H, ^{15}N−H, J ^{15}N−H = 92 Hz), 6.84 (s, 1 H), 5.10 (s, 2 H), 4.23 (q, 2 H, J = 7.2 Hz), 4.08 (m, 2 H, $^{15}NCH_2$), 4.13 (s, 3 H), 3.97 (s, 3 H), 1.85 (m, 2 H), 1.27 (t, 3 H, J = 7.2 Hz), 1.12 (t, 3 H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 12.012, 14.894, 24.756, 45.661, 49.696 ($^{15}N\mathit{C}H_2CH_2CH_3$, J ^{15}N,^{13}C = 8.5 Hz), 58.490, 62.793, 63.579, 104.534, 109.045, 111.629, 126.585, 132.946, 141.167, 142.376, 146.149 (4-C, J ^{15}N,^{13}C = 15.8 Hz), 149.966, 158.071, 168.151, 175.159.

1*H*-2,3-Diketo-4-(N-propyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (7). A solution of 29 mg (0.10 mmol) of 1*H*-2,3-diketopyrido[4,3,2-*de*]quinoline (**1**) in 20 mL of THF was mixed with 50 mg of *n*-propylamine and 5 drops of triethylamine. The mixture was stirred at room temperature for 3 h. The purple intermediate formed during the reaction was isolated immediately by flash chromatography (4:1 of ethyl acetate to hexane and then ethyl acetate) to give 22 mg of the purple 1*H*-2,3-diketo-4-(N-propyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (**7**). Yield 63%. ESMS: calcd for $C_{16}H_{16}N_3O_4Cl$ 349.1, found 350.1 (MH^+, 100%); ^{1}H NMR (DMSO-d_6): δ 11.95 (s, 1 H), 10.85 (s, 1 H), 7.23 (s, 1 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.84 (m, 2 H), 1.72 (m, 2 H), 1.02 (t, 3 H, J = 7.2 Hz).

1-(Ethoxycarbonylmethyl)-2,3-diketo-4-(N-propyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (8). A solution of 38 mg (0.10 mmol) of 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**2**) in 20 mL of THF was mixed with 50 mg of *n*-propylamine and 5 drops of triethylamine. The mixture was stirred at room temperature for 0.5 h. The purple intermediate formed during the reaction was isolated immediately by flash chromatography (1:1 of ethyl acetate to hexane and then 4:1 of ethyl acetate to hexane) to give 35 mg of the purple compound, 1-(ethoxycarbonylmethyl)-2,3-diketo-4-(N-propyl)amino-5-chloro-7,8-dimethoxypyrido-[4,3,2-*de*]quinoline (**8**). Yield 80%. ESMS: calcd for $C_{20}H_{22}N_3O_6$-Cl 435.1, found 436.1 (MH^+, 100%); ^{1}H NMR ($CDCl_3$): δ 11.58 (s, 1 H), 6.82 (s, 1 H), 5.08 (s, 2 H), 4.234 (q, 2 H, J = 7.2 Hz), 4.06 (m, 2 H), 4.10 (s, 3 H), 3.97 (s, 3 H), 1.84 (m, 2 H), 1.26 (t, 3 H, J = 7.2 Hz), 1.12 (t, 3 H, J = 7.2 Hz).

The syntheses of compound **9**−**12** were carried out by the similar manner of compound **7** and **8**. These compounds were isolated from the reaction solution as soon as possible and identified by ^{1}H NMR spectra and electrospray mass spectra. They are not stable in the solution especially in the basic solution.

1*H*-2,3-Diketo-4-amino-5-chloro-7,8-dimethoxypyrido-[4,3,2-*de*]quinoline (13). A solution of 29 mg (0.10 mmol) of 1*H*-2,3-diketopyrido[4,3,2-*de*]quinoline (**1**) in 20 mL of THF was mixed with 50 mg of *n*-propylamine and 5 drops of triethylamine. The mixture was stirred at room temperature for 24 h. The resulting solution was then subjected to column chromatography (5:1 of ethyl acetate to methanol) to give 26 mg of the red product, 1*H*-2,3-diketo-4-amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (**13**). Yield 85%, mp > 300 °C. HRMS: calcd for $C_{13}H_{10}N_3O_4Cl$ 307.0360, found 307.0361 (M^+, 100%); ^{1}H NMR (DMSO-d_6): δ 11.92 (s, 1 H), 9.57 (s, 1 H), 8.28 (s, 1 H), 7.20 (s, 1 H), 3.90 (s, 3 H), 3.88 (s, 3 H). Anal. Calcd for $C_{13}H_{10}N_3O_4Cl$: C, 50.75; H, 3.28; N, 13.66; Cl, 11.52. Found C, 51.03; H, 3.43; N, 13.78; Cl, 11.83.

1-(Ethoxycarbonylmethyl)-2,3-diketo-4-amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (14). A solution of 38 mg (0.10 mmol) of 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**2**) in 20 mL of THF was mixed with 50 mg of *n*-propylamine and 5 drops of triethylamine. The mixture was stirred at room temperature for 24 h. The resulting solution was then subjected to column chromatography (1:1 of ethyl acetate to hexane and then 4:1 of ethyl acetate to hexane) to give 36 mg of the red product, 1-(ethoxycarbonylmethyl)-2,3-diketo-4-amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (**14**). Yield 91%, mp 266−7 °C. HRMS: calcd for $C_{17}H_{16}N_3O_6Cl$ 393.0727, found 393.0726 (M^+, 100%); ^{1}H NMR ($CDCl_3$): δ 9.83 (s, 1 H), 6.90 (s, 1 H), 6.33 (s, 1 H), 5.10 (s, 2 H), 4.27 (q, 2 H, J = 7.2 Hz), 4.13 (s, 3 H), 3.99 (s, 3 H), 1.28 (t, 3 H, J = 7.2 Hz). Anal. Calcd for $C_{17}H_{16}N_3O_6Cl$: C, 51.85; H, 4.06; N, 10.67; Cl, 9.02. Found C, 52.18; H, 4.34; N, 10.78; Cl, 9.20.

1-(Ethoxycarbonylmethyl)-2,3-diketo-4-(N-phenyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (15). A solution of 175 mg (0.60 mol) of 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**2**) in 50 mL of chloroform was placed in a three-neck reaction flask which was connected with a volumeter system. The reaction system was filled with air and sealed with water in a U-type tube. Under atmospheric pressure (745 mmHg) at 23 °C, the solution was injected with 0.5 mL of triethylamine followed by 0.5 mL of aniline. The resulting mixture was stirred at 23 °C under atmospheric pressure for 24 h, and the difference of the volume in the reaction atmosphere was measured as 7.4 mL which is equivalent to 0.299 mol of oxygen (O_2). The reaction solution was washed with 1 N of hydrochloric acid (50 mL × 2) and then subjected to column chromatography (1:1 of ethyl acetate to hexane and then 4:1 of ethyl acetate to hexane) to give 160 mg of the purple product, 1-(ethoxycarbonylmethyl)-2,3-diketo-4-(N-phenyl)amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (**15**). Yield 57%, mp 205−7 °C. HRMS: calcd for $C_{23}H_{20}N_3O_6Cl$ 469.1041, found 469.1041 (M^+, 100%); ^{1}H NMR ($CDCl_3$): δ 12.15 (s, 1 H), 7.41 (t, 2 H, J = 7.4 Hz), 7.30 (t, 1 H, J = 7.4 Hz), 7.18 (d, 2 H, J = 7.4 Hz), 6.92 (s, 1 H), 5.15 (s, 2 H), 4.32 (q, 2 H, J = 7.2 Hz), 4.16 (s, 3 H), 4.02 (s, 3 H), 1.34 (t, 3 H, J = 7.2 Hz). Anal. Calcd for $C_{23}H_{20}N_3O_6Cl$: C, 58.79; H, 4.29; N, 8.94; Cl, 7.55. Found C, 58.68; H, 4.41; N, 9.21; Cl, 7.88.

1-(Ethoxycarbonylmethyl)-2,3-diketo-4-[N-(3,4-dimethoxy)phenyl]amino-5-chloro-7,8-dimethoxypyrido-[4,3,2-*de*]quinoline (16). A solution of 38 mg (0.10 mmol) of 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]quinoline (**2**) in 20 mL of chloroform was mixed with 100 mg of 3,4-dimethoxyaniline and 10 drops of triethylamine. The mixture was stirred at room temperature for 24 h. The resulting solution was washed with 1 N of hydrochloric acid (50 mL × 2) and then subjected to column chromatography (1:1 of ethyl acetate to hexane and then 4:1 of ethyl acetate to hexane) to

give 40 mg of the purple product, 1-(ethoxycarbonylmethyl)-2,3-diketo-4-[*N*-(3,4-dimethoxy)phenyl]amino-5-chloro-7,8-dimethoxypyrido[4,3,2-*de*]quinoline (**16**). Yield 75%, mp 225−7 °C. HRMS: calcd for $C_{25}H_{24}N_3O_8Cl$ 529.1252, found 529.1229 (M^+, 100%); ^1H NMR ($CDCl_3$): δ 12.27 (s, 1 H), 6.94 (s, 1 H), 6.90 (d, 1 H, J = 6.3 Hz), 6.70−6.80 (m, 2 H), 5.12 (s, 2 H), 4.30 (q, 2 H, J = 7.2 Hz), 4.14 (s, 3 H), 4.01 (s, 3 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 1.31 (t, 3 H, J = 7.2 Hz). Anal. Calcd for $C_{25}H_{24}N_3O_8Cl$: C, 56.66; H, 4.56; N, 7.93; Cl, 6.69. Found C, 56.68; H, 4.38; N, 8.21; Cl, 6.94.

Compound **16** can be crystallized from 4:1 of ethyl acetate to hexane to give single crystals.

Reaction of Hydroxylamine or Hydrazine with 2,3-Diketopyrido[4,3,2-*de*]quinolines. A solution of 38 mg (0.10 mmol) of 1-(ethoxycarbonylmethyl)-2,3-diketopyrido[4,3,2-*de*]-quinoline (**2**) in 20 mL of THF was mixed with 100 mg of hydroxylamine hydrochloride (or hydrazine monohydrate) and 10 drops of triethylamine. The mixture was stirred at room temperature for 24 h. A red product formed in the solution. After removing the solvent, the residue was extracted with chloroform (30 mL × 2) and subjected to the column chromatography (1:1 of ethyl acetate to hexane and then 4:1 of ethyl acetate to hexane and then ethyl acetate). The red product was identified by mp, ^1H NMR, and HRMS. All of these analytical results demonstrate that this red product is identical with compound **14**.

Reaction of <bold>α**-Amino Acids with 2,3-Diketopyrido[4,3,2-*de*]quinoline 1.** A solution of 29 mg (0.10 mmol) of 1*H*-2,3-diketopyrido[4,3,2-*de*]quinoline (**1**) was suspended in 30 mL of water whose pH value was adjusted to 10 with triethylamine, and 76 mg (10 mmol) of glycine was added. The resulting mixture was stirred at room temperature for 72 h. After neutralizing with diluted hydrochloric acid, the precipitate was collected by filtration and washed with water. The results of ^1H NMR and HRMS show that this precipitate is the pure compound of 4-amino-2,3-diketopyrido[4,3,2-*de*]quinoline (**13**).

Reaction of Substance P with 2,3-Diketopyrido[4,3,2-*de*]quinoline 1. A solution of 10 μL of 1.2 mM Substance P and 10 μL of 10 mM of 1*H*-2,3-diketopyrido[4,3,2-*de*]quinoline **1** in DMSO were mixed in 1 mL of water whose pH value was adjusted to 9 with triethylamine. The resulting mixture was stirred at 37 °C and subjected to MALDI mass and electrospray mass analysis at 10 min, 3.5 h, and 3 days. Samples were typically prepared by using the two-layer method.[40] The first layer was formed by depositing 1 μL of 12 mg/mL matrix material in acetone on the probe,[41] followed by addition of 1 μL of standards or sample mixed with matrix. MALDI MS: calcd for **19** 1654.3, found 1655.1 (MH^+). ESMS: calcd for **19** 1654.3, found 1655.7 (MH^+).

Acknowledgment. We gratefully thank the Natural Sciences and Engineering Research Council of Canada for financial support of this work (to J.W.L.).

Supporting Information Available: ORTEP drawings for **16**. Crystallographic experimental details, atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, torsional angles, anisotropic displacement parameters, derived atomic coordinates, and displacement parameters for hydrogen atoms. Two ^1H NMR spectra for ^{15}N-labeled compound **4** and **6** and one ^{13}C NMR spectrum for ^{15}N-labeled compound **6**.

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