

## Total Synthesis of the Antifungal Dilactones UK-2A and UK-3A: The Determination of their Relative and Absolute Configurations, Analog Synthesis and Antifungal Activities.

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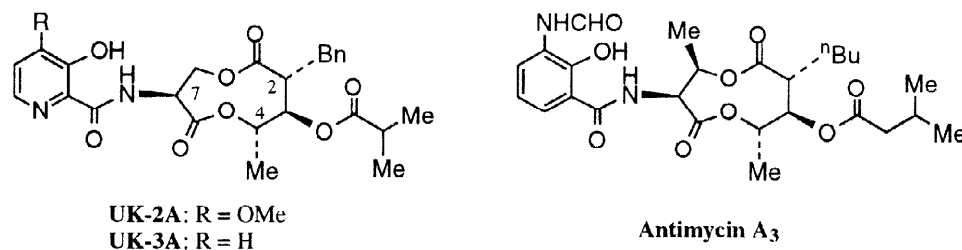
**Abstract** The synthesis of the antifungal dilactones, UK-2A and UK-3A, is described. In addition to providing a workable synthetic route to these potent antifungal antibiotics, this has allowed us to determine the assignment of the relative and absolute configurations in the nine-membered ring. Furthermore, UK-2A analogs were also synthesized and evaluated their antifungal activities and cytotoxic activities along with UK-2A, (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A, UK-3A, (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A, and antimycin A. The structural requirements for the selective cytotoxicity against yeasts and filamentous fungi will also be suggested. © 1998 Elsevier Science Ltd. All rights reserved.

**Keywords:** Antifungals; Asymmetric synthesis; Medium-ring heterocycles; Mitsunobu reactions.

The research of the antifungal antibiotics, especially the agents for the treatment of systematic fungal infections, has become one of the major concerns in medicinal chemistry because of the lack of a specific remedy and a great need of new antifungal agents.<sup>1,2,3</sup> Although the lack of lead compounds which are essentially new based upon the mechanism of antifungal activities<sup>3,4</sup> has retarded progress in this field, several kinds of compounds are now being examined in clinical trials.<sup>1</sup>

In our continuous study toward the development of new antifungal agents, we have been interested in the naturally occurring products, UK-2A and UK-3A. UK-2A is a nine-membered dilactone which has recently been isolated along with the structurally similar congeners, UK-2B, 2C and 2D, from the mycelial cake of *Streptomyces* sp. 517-02 by Taniguchi *et al.*<sup>5</sup> The plane structure of UK-2A has been elucidated by detailed <sup>1</sup>H and <sup>13</sup>C NMR analyses and chemical degradation studies, but the relative and absolute configurations of the four chiral centers in UK-2A still remain to be determined.<sup>6</sup> The structure of UK-2A is apparently similar to the well-known antimycins,<sup>7</sup> but the benzyl group at the C<sub>2</sub> position in UK-2A has never existed in known antimycins<sup>8</sup> and one methyl group is lacking at the C<sub>8</sub> position. Furthermore, UK-2A has a 3-hydroxy-4-methoxypyridine-

2-carbonyl group which had never been found in naturally occurring products, while the antimycins have instead the 3-formamidosalicylyl group which is believed essential to blocking the electron flow in the mitochondrial respiratory chain between cytochromes b and c<sub>1</sub>.<sup>9</sup> Another, and the most striking difference between them, is their biological activities. UK-2A has strongly inhibited the growth of various kinds of yeasts and filamentous fungi, but the cytotoxic activities against several kinds of mammalian cells was very weak, while the antimycins have inhibited mammalian cells as strongly as fungi.<sup>5a</sup> The basicity of the pyridine ring should make UK-2A considerably more basic than the antimycins so that this physical difference may contribute to the remarkable change in its biological activities. UK-3A has also recently been isolated from the mycelial cake of *Streptomyces* sp. 517-02 and its plane structure was determined on the basis of the correlation with UK-2A while the relative and absolute configurations have not yet been determined.<sup>10</sup> It has antifungal activities similar to UK-2A. From these facts, we have considered UK-2A and UK-3A as attractive targets for asymmetric synthesis, and at the same time, as the potential antifungal agents. Recently, we reported in a preliminary manner, the enantioselective total synthesis of UK-2A.<sup>11</sup> We now wish to describe the details of the total synthesis of UK-2A and UK-3A in which the relative and absolute configurations of the four asymmetric centers in the nine-membered dilactone is unequivocally determined using a well-established asymmetric reaction. The reported degradation products will be also discussed in order to reconfirm the configurations of UK-2A. In the last part of this full account, we will refer to the analog synthesis of UK-2A, their antifungal activities and cytotoxic activities, and the structural demands for the selective cytotoxicity against yeasts and filamentous fungi will also be briefly described.



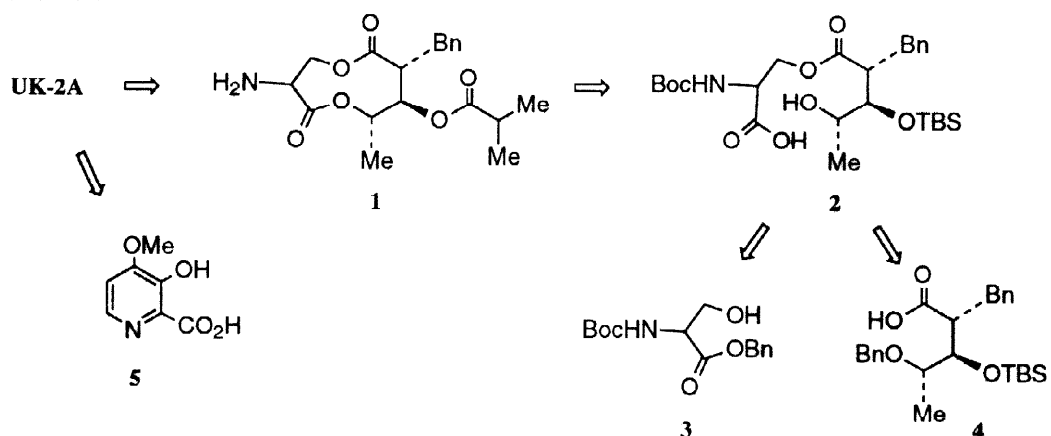
## Enantioselective Total Synthesis of UK-2A

### *Synthesis of Nine-membered Dilactone*

As the relative configuration of the three consecutive chiral centers from C<sub>2</sub> to C<sub>4</sub> has been determined as (2*R*, 3*R*, 4*S*) or its antipode, we decided to synthesize the two diastereomers, (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A.

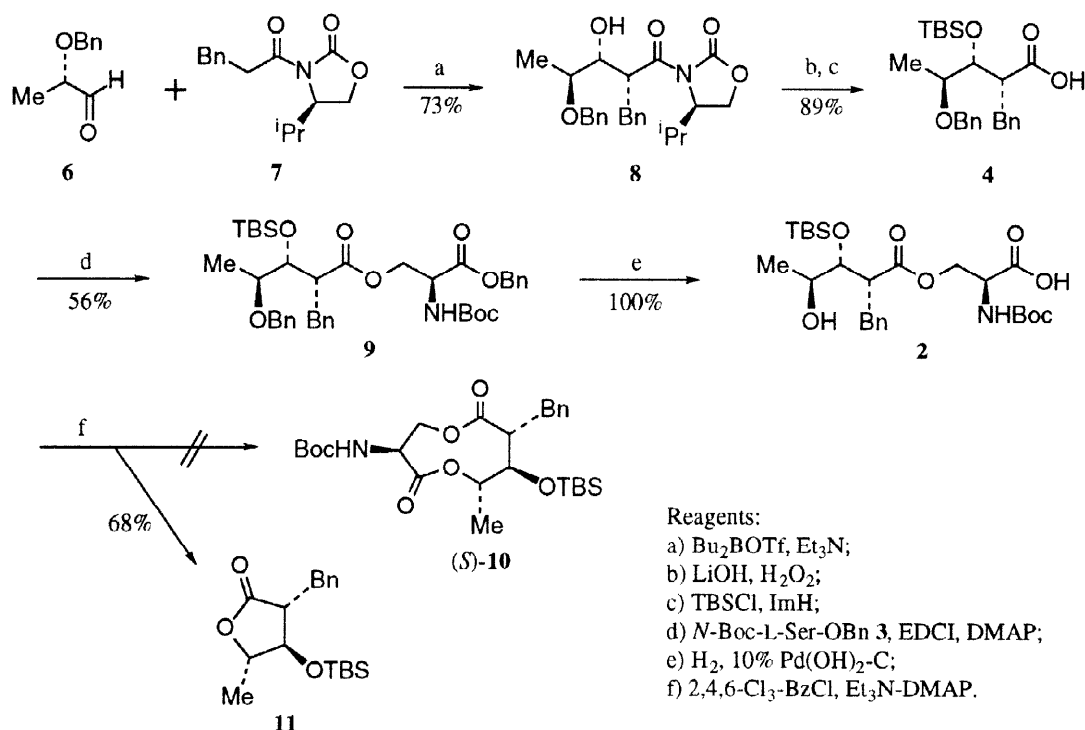
Our first synthetic strategy is illustrated in Scheme 1, where the key intermediates were the nine-membered dilactone **1** and 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**). The nine-membered dilactone **1** would be synthesized from the corresponding seco acid by the lactonization reaction in which the cyclization occurred between the carboxy group in the serine moiety and the hydroxy group at the C<sub>4</sub> position in the pentanoic acid moiety. The seco acid **2** would be prepared from the L-/D-serine derivative **3** and an optically pure 3,4-dihydroxypentanoic acid derivative **4** which should be obtained by means of the well-established asymmetric reaction for the purpose of determining the stereochemistry of the targets. The synthesis of 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) will be discussed in the next section.

## Scheme 1



The synthesis of the optically pure 3,4-dihydroxypentanoic acid **4** was achieved through the asymmetric Evans aldol reaction<sup>12</sup> between the aldehyde **6**<sup>13</sup>, and *N*-hydrocinnamoyloxazolidinone **7**,<sup>14</sup> prepared from hydrocinnamoyl chloride and (*R*)-4-isopropylloxazolidin-2-one (78%) (Scheme 2). The aldol reaction occurred with high diastereoselectivity (>98% de) to provide after column chromatography alcohol **8** as a single diastereomer in 73% yield. In order to prepare the cyclization precursor **2**, the chiral auxiliary was first removed with LiOH/H<sub>2</sub>O<sub>2</sub><sup>15</sup> to give rise to the free acid and the following *t*-butyldimethylsilyl protection of the 3-hydroxy group afforded **4** in 89% yield. The ester formation with the suitably protected L-serine derivative **3** in the presence of EDCI/DMAP yielded the protected seco acid **9** in 56% yield. The hydrogenation under H<sub>2</sub> with a Pd-C catalyst removed the two benzyl groups at the same time and we could obtain the desired seco acid **2** in quantitative yield. The seco acid **2** was then subjected to Yamaguchi's cyclization procedure.<sup>16</sup>

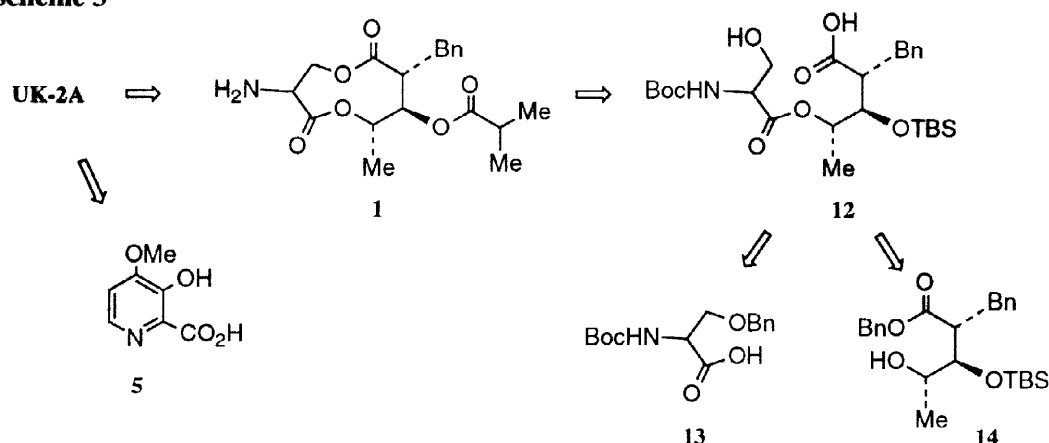
## Scheme 2



After several attempts by varying the reaction conditions, we had to admit that the main product was always the  $\gamma$ -lactone **11** instead of the desired nine-membered dilactone (*S*)-**10**. Once the  $\gamma$ -lactone **11** was formed, it was quite difficult to change this  $\gamma$ -lactone to reproduce the usable compound. Furthermore, it turned out that the seco acid **2** gradually decomposed into the  $\gamma$ -lactone **11** at room temperature. Though there might be a possibility of other kinds of lactonization reactions going well, we abandoned this strategy for a new one.

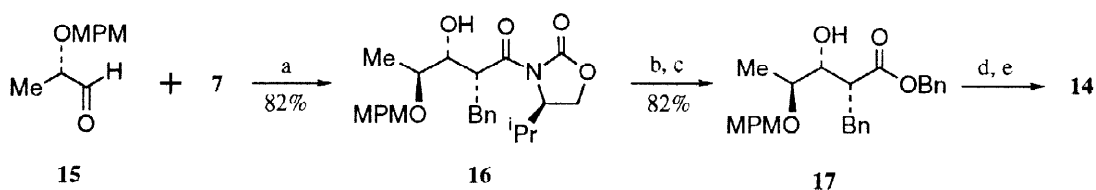
Our second synthetic strategy for the nine-membered dilactone formation is depicted in Scheme 3. In this strategy, the key intermediate **1** was synthesized from the seco acid **12** by the lactonization reaction in which another pair of hydroxy group and carboxy group were used other than the one in the former strategy in order to avoid the  $\gamma$ -lactone formation.

Scheme 3



The asymmetric Evans aldol reaction between the aldehyde **15**, prepared in two steps from ethyl (*S*)-(-)-lactate by *p*-methoxybenzylation<sup>17</sup> and the DIBAL reduction (67%), and *N*-hydrocinnamoyloxazolidinone **7**, lead to excellent diastereoselectivity (>98%) and afforded the alcohol **16** as a single diastereomer in 82% yield (Scheme 4). The chiral auxiliary was then replaced with benzyl alcohol in a two-step sequence.<sup>18</sup> The protection of the hydroxy group and the cleavage of the MPM group<sup>19</sup> to give alcohol **14** was carried out without incident.

Scheme 4

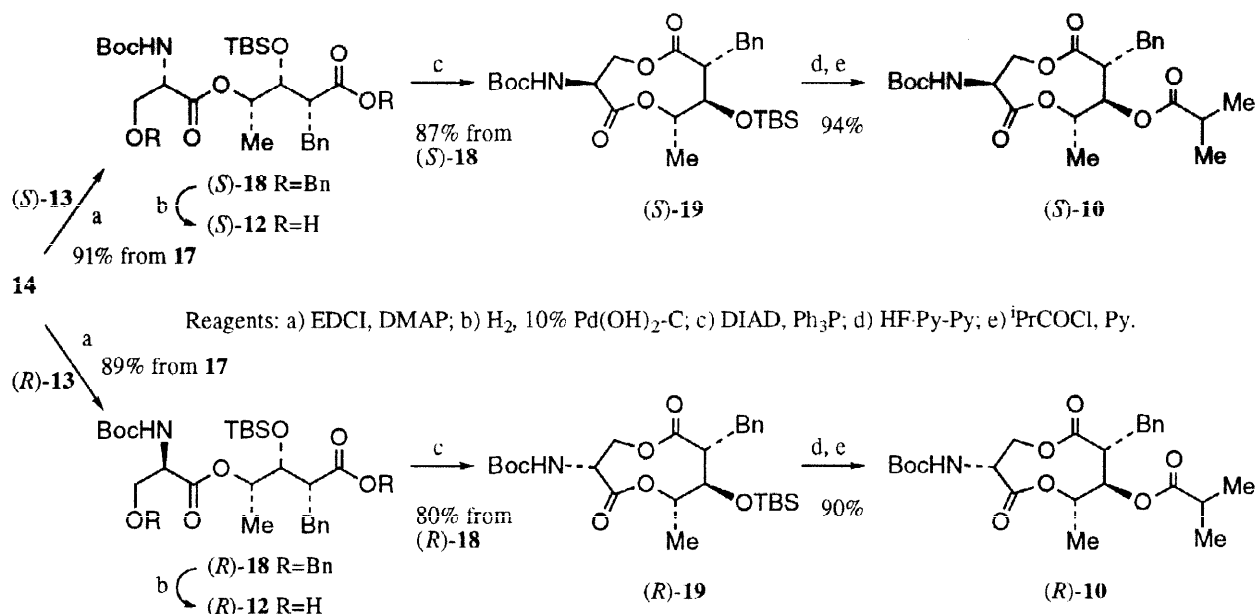


Reagents: a)  $\text{Bu}_2\text{BOTf}$ ,  $\text{Et}_3\text{N}$ ; b)  $\text{LiOH}$ ,  $\text{H}_2\text{O}_2$ ; c)  $\text{BnOH}$ ,  $\text{DIAD}$ ,  $\text{Ph}_3\text{P}$ ; d)  $\text{TBSCl}$ ,  $\text{ImH}$ ; e)  $\text{DDQ}$ ,  $\text{H}_2\text{O}$ .

The couplings of alcohol **14** with each L-serine derivative (*S*)-**13** and D-serine derivative (*R*)-**13** were conducted in the presence of EDCI/DMAP and gave (*S*)-**18** and (*R*)-**18**, respectively (Scheme 5). It should be noted that the coupling with the D-serine derivative (*R*)-**13** suffered a serious racemization at the C<sub>7</sub>-position under the normal conditions where the coupling between the alcohol **14** and L-serine derivative (*S*)-**13** went smoothly accompanied with less than 3% racemization at the C<sub>7</sub> position. However, after several trials, under the conditions using a lower reaction temperature (-10°C), we could minimize this side-reaction (>93% selectivity). The hydrogenolysis of the two benzyl group afforded the lactonization precursors, seco acids (*S*)-**12** and (*R*)-**12**.

First, Yamaguchi's method<sup>16</sup> and modified Yamaguchi's method<sup>20</sup> were examined in order to realize the nine-membered lactonization, but in either case, it turned out to be fruitless. Only a trace of cyclization product was detected along with many undetermined products. Therefore, the alternative standard, the intramolecular Mitsunobu reaction,<sup>21</sup> was conducted by the treatment of (*S*)-**12** with diisopropyl azodicarboxylate (DIAD) and Ph<sub>3</sub>P. The desired lactonization cleanly occurred and afforded dilactone (*S*)-**19** in 87% yield.<sup>22</sup> Further elaboration to functionalize the nine-membered ring required little work, and consequently, we were able to obtain (*S*)-**10** on a multi-gram scale. Another diastereomer (*R*)-**10** was also synthesized without event in the same manner as shown in Scheme 5.

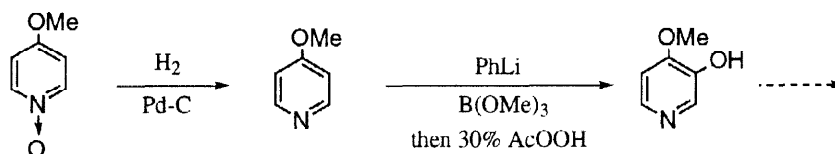
### Scheme 5



### Synthesis of 3-Hydroxy-4-methoxypyridine-2-carboxylic Acid (**5**)

We initially chose the known 3-hydroxy-4-methoxypyridine<sup>23</sup> as the starting material which could be prepared from commercial 4-methoxypyridine *N*-oxide (Scheme 6). However, 3-hydroxy-4-methoxypyridine was extremely water-soluble so that it required much work to take up it from the reaction mixture of phenyllithium-B(OMe)<sub>3</sub>-30% AcOOH. Furthermore, this reaction required explosive 30% AcOOH as an oxidant, so that it must be conducted under special conditions if prepared on a multi-gram scale.

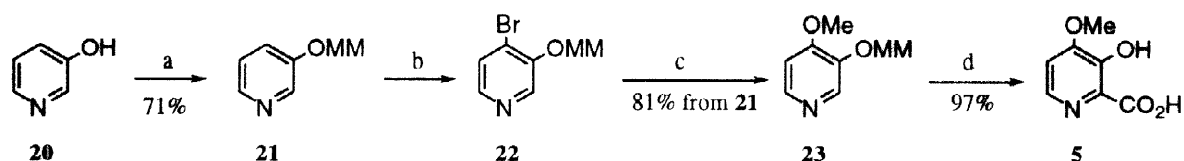
### Scheme 6



From these reasons, we changed our mind and re-selected 3-hydroxypyridine (**20**) as the new starting material (Scheme 7). The hydroxy group of 3-hydroxypyridine (**20**) was first converted to the methoxymethyl ether in order to protect the hydroxy group, and at the same time, as an *ortho* lithiation directing group. 3-

Methoxymethoxypyridine (**21**) was then deprotonated at the C<sub>4</sub>-position according to Ronald's procedure<sup>24</sup> and quenched with 1,2-dibromo-1,1,2,2-tetrafluoroethane to give rise to 4-bromo-3-methoxymethoxypyridine (**22**).<sup>25</sup> The replacement of bromine with a methoxy group was successfully carried out with NaOMe/MeOH and afforded 4-methoxy-3-methoxymethoxypyridine (**23**) in 81% yield from **21**. The carboxylation at the C<sub>2</sub>-position was then examined using several lithiating reagents under various conditions, and *t*-butyllithium in THF at -78°C followed by dry-ice quenching gave the best results (>98% regioselectivity), and acidic work-up afforded 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) in 97% yield. The total yield of 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) from the commercially available 3-hydroxypyridine (**20**) was greater than 50%.

Scheme 7

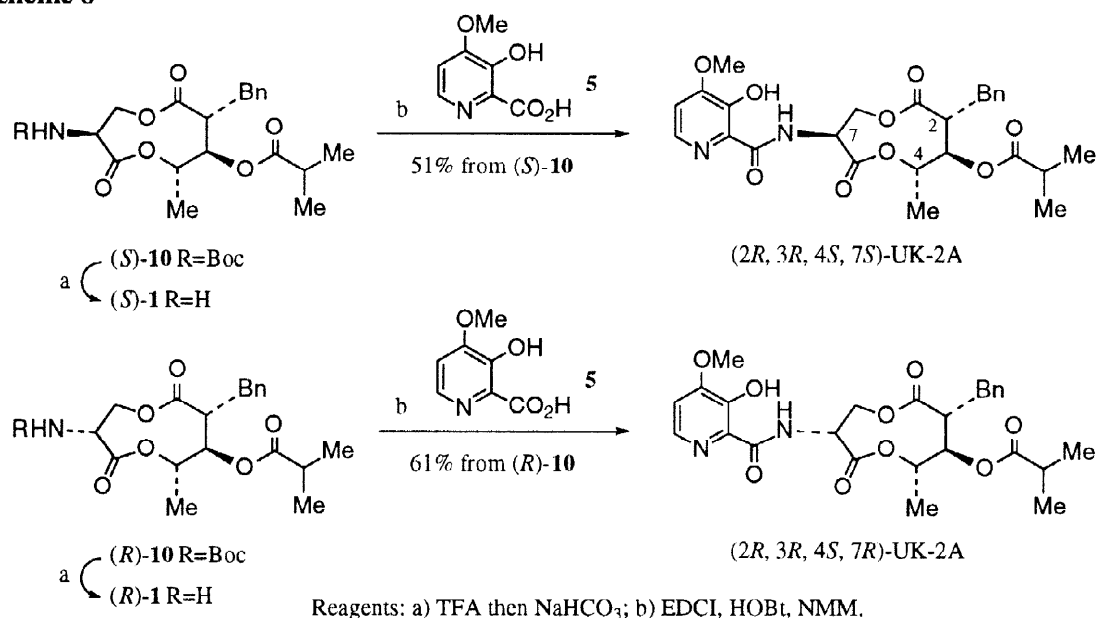


Reagents: a) MMCl, <sup>t</sup>BuOK, THF-DMF; b) <sup>t</sup>BuLi, BrCF<sub>2</sub>CF<sub>2</sub>Br, Et<sub>2</sub>O, -78°C; c) NaOMe, MeOH; d) <sup>t</sup>BuLi, CO<sub>2</sub>, THF, -78°C, then aq.HCl.

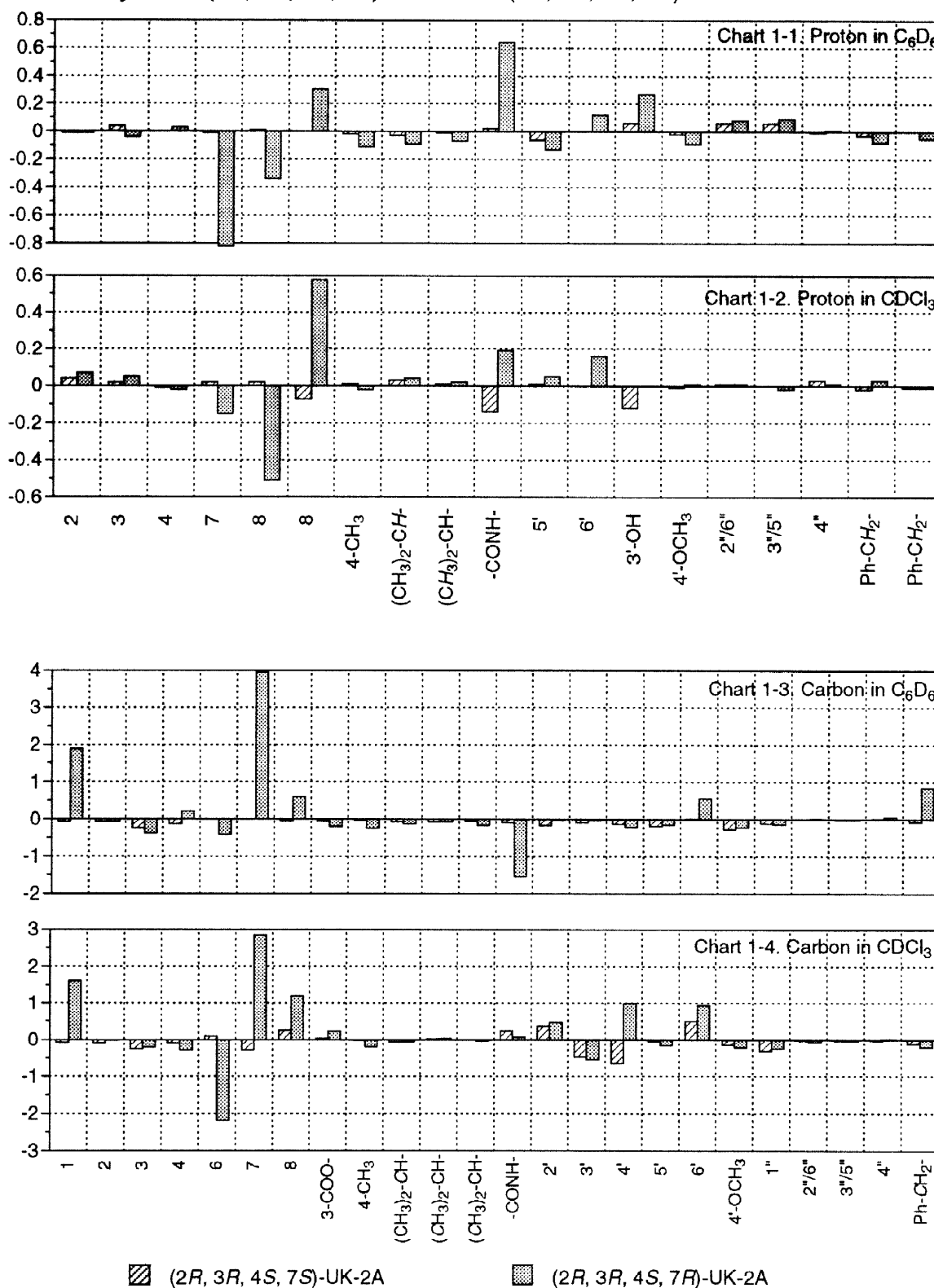
### Enantioselective Total Synthesis of UK-2A

The final stage in the total synthesis of UK-2A, the coupling of 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) with each dilactone (*S*)-**1** and (*R*)-**1** prepared from each of the corresponding Boc derivatives (*S*)-**10** and (*R*)-**10** by TFA-CH<sub>2</sub>Cl<sub>2</sub>, was successfully achieved in the presence of EDCI/HOBt, respectively (Scheme 8). Both (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A were subjected to <sup>1</sup>H and <sup>13</sup>C NMR studies. Chart 1 shows the difference in the chemical shifts of protons and carbons in C<sub>6</sub>D<sub>6</sub> between natural UK-2A and each synthesized diastereo UK-2A (Charts 1-1 and 1-3).

Scheme 8



**Chart 1.** Difference in Proton and Carbon Chemical Shifts between Reported UK-2A and each of Synthetic (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A.



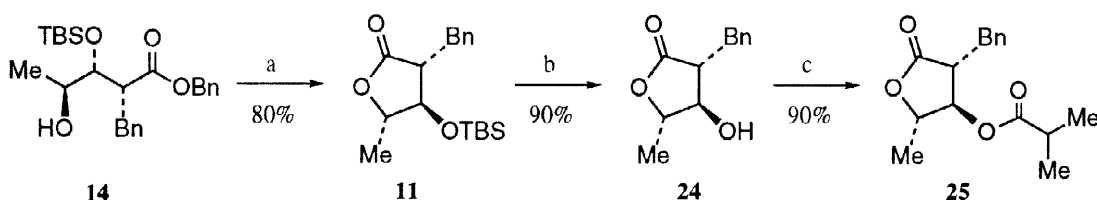
The y-axes represent  $\delta\Delta$  ( $\delta\Delta = \delta_{\text{synthetic}} - \delta_{\text{natural}}$  in ppm)

Other NMR experiments in  $\text{CDCl}_3$  are also shown in the chart (Charts 1-2 and 1-4). These exercises clearly demonstrated that (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A exhibited distinct spectroscopic characteristics that differed from each other, and that (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A displayed spectroscopic characteristics that were identical to those of natural UK-2A, therefore, establishing that the relative stereochemistry of the four asymmetric centers in the dilactone of UK-2A is (2*R*, 3*R*, 4*S*, 7*S*) or its antipode. The specific rotation of synthesized (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A was then measured under the same conditions in which natural UK-2A was made, and (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A had virtually an identical specific rotation with natural UK-2A  $\{[\alpha]^{23}_{\text{D}} +89.29$  (*c* 1.01,  $\text{CHCl}_3$ ); lit.  $[\alpha]^{23}_{\text{D}} +89.11$  (*c* 0.8,  $\text{CHCl}_3$ )<sup>5</sup>. Therefore we were able to achieve the first total synthesis of UK-2A in an optically pure form, and at the same time, the absolute configurations in the dilactone of UK-2A was unequivocally determined as (2*R*, 3*R*, 4*S*, 7*S*).<sup>26</sup>

### The Synthesis of the Reported Degradation Products of UK-2A

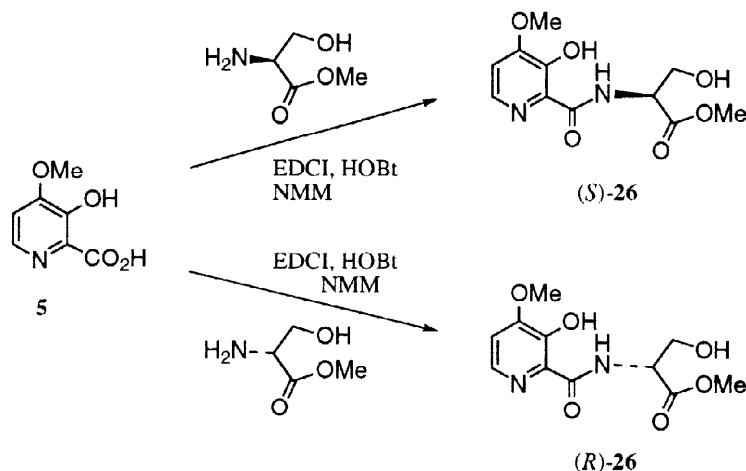
In order to reconfirm the configurations of UK-2A, we synthesized the reported degradation products<sup>5</sup> of UK-2A,  $\gamma$ -lactone **24**, **25**, both enantiomers of picolinylserine methyl ester (*S*)-**26** and (*R*)-**26**, in optically pure forms (Schemes 9 and 10). The physical data of the synthetic  $\gamma$ -lactone **25** were identical with the reported  $\gamma$ -lactone **25**, so that we could reconfirm the absolute stereochemistry of the three consecutive asymmetric centers from C<sub>2</sub> to C<sub>4</sub> as (2*R*, 3*R*, 4*S*).<sup>27</sup>

Scheme 9



Reagents: a) room temperature; b) HF·Py·Py; c) <sup>1</sup>PrCOCl, Py.

Scheme 10



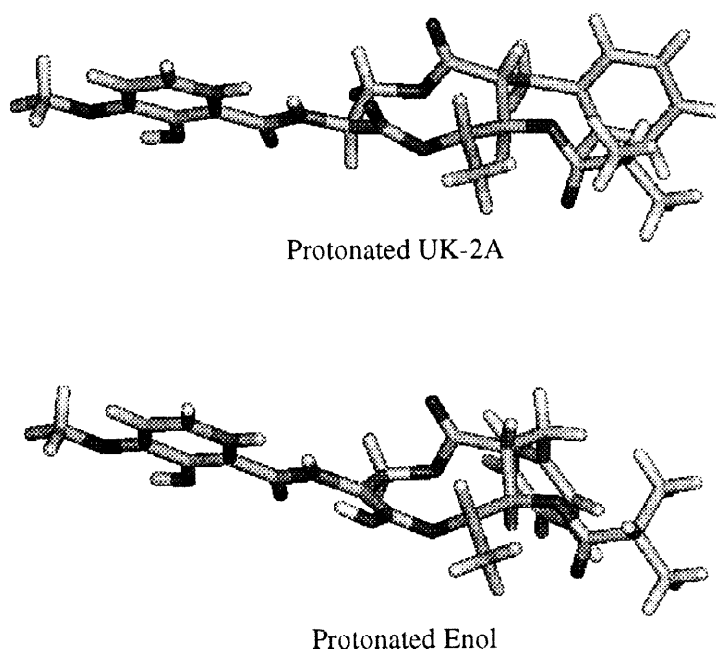
The absolute configuration of the reported picolinylserine methyl ester **26** was confusing. The <sup>1</sup>H and <sup>13</sup>C NMR of both enantiomers of the picolinylserine methyl ester **26** synthesized were identical with that of the

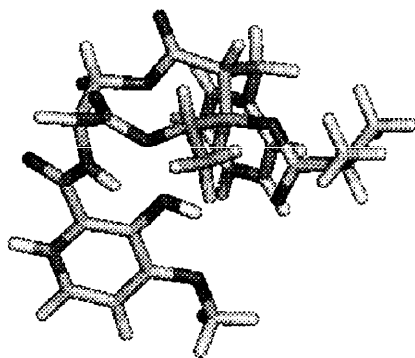


reported picolinylserine methyl ester **26**. The synthesized picolinyl-L-serine methyl ester **26**, which has the *S* configuration, has shown  $[\alpha]^{27}_{\text{D}} +29.0^{\circ}$  ( $c$  0.44,  $\text{CHCl}_3$ ), and the synthesized picolinyl-D-serine methyl ester **26**, which has the *R* configuration, has shown  $[\alpha]^{27}_{\text{D}} -29.72$  ( $c$  1.01,  $\text{CHCl}_3$ ). On the other hand, the reported picolinylserine methyl ester **26** has shown  $[\alpha]^{27}_{\text{D}} -11.03$  ( $c$  0.60,  $\text{CHCl}_3$ ). From these results, it appeared that the reported picolinylserine methyl ester **26** was not optically pure and that the picolinyl-D-serine methyl ester (*R*)-**26** was a major composer of it. This result was not in accordance with the absolute configuration at the C7 position of UK-2A, because UK-2A has been synthetically determined to have the *S* configuration at the C7 position. The specific rotation of the reported picolinylserine methyl ester **26** has suggested that the epimerization at the C7 position took place during the acidic methanolysis and this is not unusual, but that the major composer being the picolinyl-D-serine methyl ester (*R*)-**26** is somewhat strange. This has meant that before UK-2A was methanolized into the picolinylserine methyl ester (*S*)-**26**, the C7 position of UK-2A was epimerized, and at the same time, (*2R, 3R, 4S, 7R*)-UK-2A appeared to be thermodynamically more stable than natural (*2R, 3R, 4S, 7S*)-UK-2A under the methanolized conditions. An alternative way to think of it is that (*2R, 3R, 4S, 7R*)-UK-2A underwent methanolysis much faster than (*2R, 3R, 4S, 7S*)-UK-2A and that the equilibration consequently increased the amount of the picolinyl-D-serine methyl ester (*R*)-**26**.

In order to elucidate why the D-serine form became dominant during the acidic degradation conditions, we performed the conformational searches on both (*2R, 3R, 4S, 7S*)-UK-2A and (*2R, 3R, 4S, 7R*)-UK-2A and their common enol tautomer at the C7 position<sup>28</sup> in a molecular mechanics basis (Quanta/CHARMM).<sup>29</sup> The enol form was supposed to be the initial transition state during the course of the epimerization. The search strategy using the iterative computations of MD trajectories at high (900K) and low (300K) temperatures was completely the same as the reported procedure by Perez et al.<sup>30</sup> As shown in Figure 1, the overlay of the lowest potential energy conformations of each one indicated that their ring conformations were almost identical within a 0.27 rms difference value. Moreover, the conformations of (*2R, 3R, 4S, 7S*)-UK-2A and the enol were quite similar to each other including the side-chain at the C7 position, while the conformation of (*2R, 3R, 4S, 7R*)-UK-2A had to have an axial array of the side-chain at the C7 position.

Figure 1



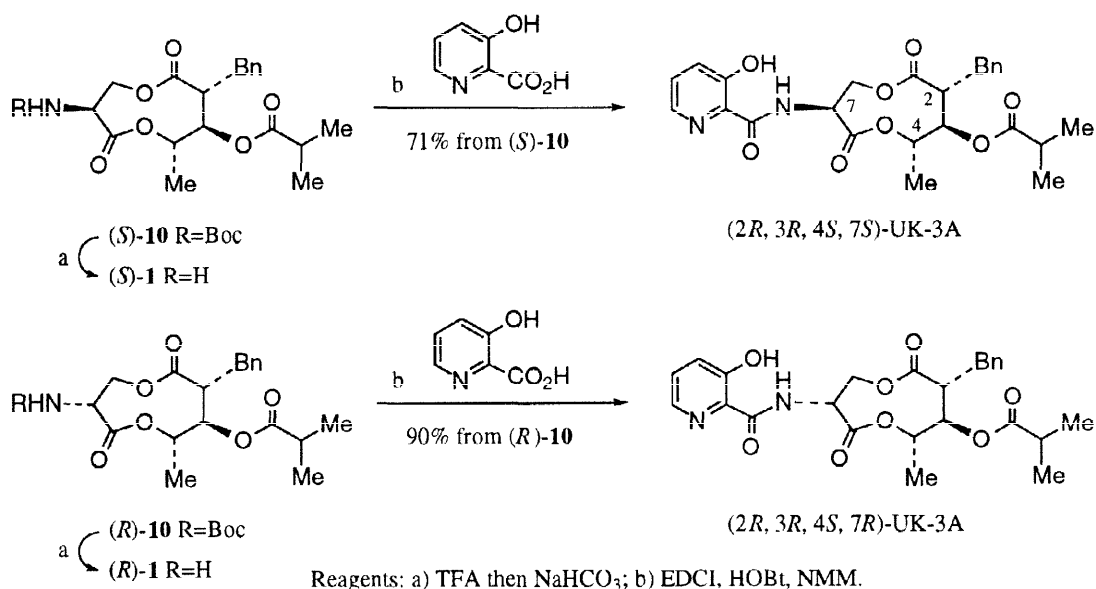
Protonated (7*R*)-UK-2A

This has clearly meant that (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A has already the much closer conformation to its enol form, namely the transition state, in the low conformation energy level than has (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A. From a thermodynamic point of view, this would be quite reasonable for (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A to be favorably epimerized and then dominantly changed to (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A.<sup>31</sup> Therefore, the epimerization experiments were examined under acidic conditions in an aprotic solvent so that UK-2A was intended not to be solvolyzed into smaller molecules. 4*N* HCl in dioxane at between 0°C and 60°C did not do anything with UK-2A. UK-2A was completely stable under these conditions. TFA-CH<sub>2</sub>Cl<sub>2</sub> was then used, but no reaction was observed. Sulfuric acid was then tried, but in this case, UK-2A instantly disappeared and a very polar complex mixture was obtained. Based on these results, it seemed to be rather difficult to obtain (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A under acidic conditions. At that time, we abandoned our pursuit of the epimerization.

### Enantioselective Total Synthesis of UK-3A

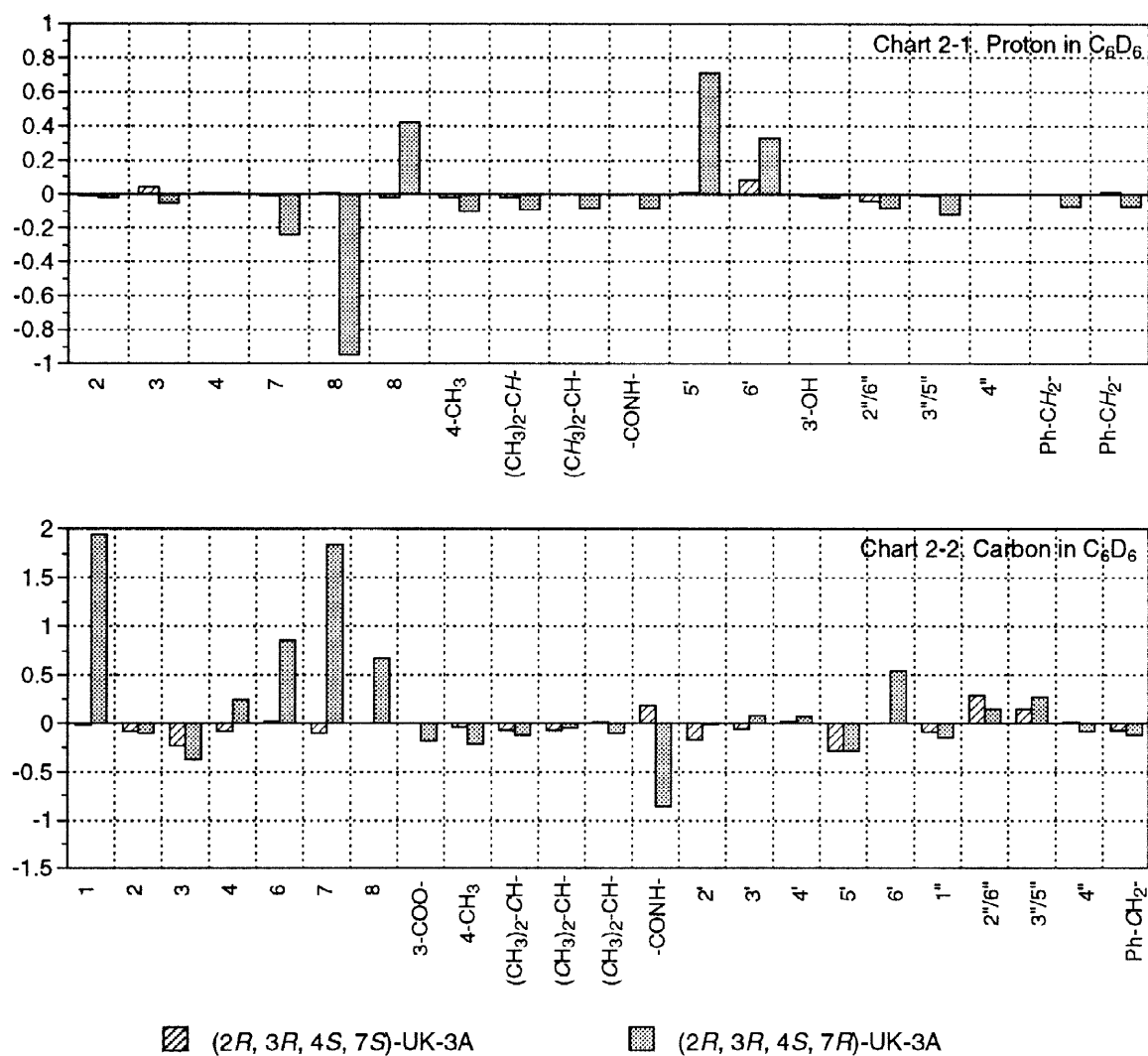
Both (2*R*, 3*R*, 4*S*, 7*S*)-UK-3A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A were synthesized from the corresponding precursors respectively as shown in Scheme 11.

#### Scheme 11



Both (2*R*, 3*R*, 4*S*, 7*S*)-UK-3A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A were subjected to <sup>1</sup>H and <sup>13</sup>C NMR analyses. Chart 2 shows the difference in the chemical shifts of protons and carbons between natural UK-3A and each synthesized diastereo UK-3A. This exercise clearly demonstrated that (2*R*, 3*R*, 4*S*, 7*S*)-UK-3A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A exhibited distinct spectroscopic characteristics differing from each other, and that (2*R*, 3*R*, 4*S*, 7*S*)-UK-3A displayed spectroscopic characteristics that were identical to those of natural UK-3A, therefore, establishing that the relative stereochemistry of the four asymmetric centers in the dilactone of UK-3A is (2*R*, 3*R*, 4*S*, 7*S*) or its antipode.<sup>32</sup> As the specific rotation of natural UK-3A has not been reported, there is no means to determine the absolute stereochemistry of UK-3A. But we believe that UK-3A has the same absolute configuration with UK-2A because of the fact that UK-3A was isolated from the same mycelial cake with UK-2A.

**Chart 2.** Difference in Proton and Carbon Chemical Shifts between Reported UK-3A and each of Synthetic (2*R*, 3*R*, 4*S*, 7*S*)-UK-3A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A.

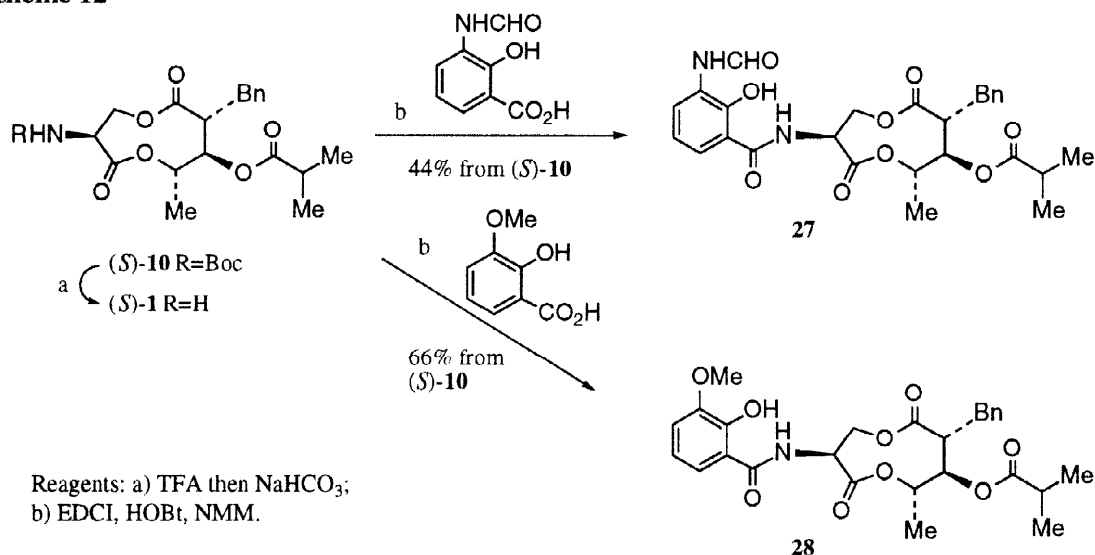


The y-axes represent  $\delta\Delta$  ( $\delta\Delta = \delta_{\text{synthetic}} - \delta_{\text{natural}}$  in ppm)

### Synthesis of UK-2A Analogs and Their Antifungal Activities and Cytotoxic Activities

UK-2A has been reported to have a very strong and broad antifungal spectrum like antimycin A<sub>3</sub>, but its cytotoxic activities against several mammalian cells are very weak compared to those of antimycin A.<sup>5a</sup> Therefore, there should be a large difference between them. In order to define this difference, the two analogs of UK-2A were synthesized (Scheme 12). One analog was a hybrid between UK-2A and antimycin A, which had the 3-formamidosalicylyl group, and the other was the 3-methoxysalicylic acid analog which seemed to have the same steric environment around the aromatic carboxamide with UK-2A but the basicity of the molecule could be much lower than UK-2A.

Scheme 12



The susceptibility of a variety of yeasts and filamentous fungi to these two analogs, **27** and **28**, along with UK-2A, (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A, UK-3A, (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A, and antimycin A was determined compared with the reference compounds, amphotericin B and fluconazole, by the serial 2-fold agar dilution method according to the reported procedure.<sup>5a</sup> As summarized in Table 1, UK-2A, antimycin A and the hybrid analog **27**, have a broad antifungal spectrum. It should be noted that these three compounds were active against the *Aspergillus* sp. which often shows little susceptibility toazole antifungal agents. On the other hand, it was a quite surprising result that none of the tested compounds were unable to affect the *Candida* sp. in Table 1. Since the 3-methoxysalicylic acid analog **28** appeared to have no antifungal activity against the tested microorganisms on the list in Table 1, the nitrogen in the picolinic acid moiety seemed to be essential to having antifungal activity. Perhaps the basicity of the nitrogen changes the permeability and/or the behavior of the molecule in the microorganism cells. Unexpectedly, the C<sub>7</sub> epimer, (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A showed no activity against the tested microorganisms. This result suggested that the configuration at the C<sub>7</sub> position was very important for the antifungal activity. Therefore, we carried out a conformational search on antimycin A<sub>3</sub> to obtain a rough idea about the bioactive conformation of these series of compounds. Although antimycin A<sub>3</sub> has an extra methyl group at the C<sub>8</sub> position and it would be postulated that this should result in a different conformational space distribution on the ring conformation from UK-2A, UK-2A and antimycin A<sub>3</sub> can be imagined to share the same type of total shape to bind to the same kind of receptor based upon the antifungal activities. Consequently, both UK-2A and antimycin A<sub>3</sub> turned out to have a very similar "extended (all equatorial form)" shape in a low energy

conformation for each even though the "extended" form could not be sampled at all within 3 kcal/mol from the lowest energy level in regard to (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A. Although we understand that more SAR data should be needed for further pharmacophore development, it can be said that the total shape of these molecules would be important for the antifungal activities.

UK-3A was only able to affect the *Trichophyton* sp. at rather high concentrations and its C7 epimer, (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A, had no antifungal activity at all in our experiment. This suggested that the methoxy group at the C4' position was very important for the antifungal activity.

The cytotoxic activities of all tested compounds were also examined against human embryonic lung fibroblast (HEL) cells, mouse leukemia (P-388) cells and mouse lymphoma (EL-4) cells. These results are summarized in Table 2. Amphotericin B and antimycin A showed strong cytotoxicities against P-388 and EL-4, but UK-2A had a very weak cytotoxicity against all tested cell lines. As the hydrophobicity of UK-2A is greatly reduced by the presence of the pyridine ring compared to that of antimycin A, this difference may reflect the performance of UK-2A during transceller absorption. The hybrid analog **27** showed relatively weak cytotoxicities compared to antimycin A. It should be stated that the hybrid analog **27** has the same 3-formamidosalicylyl group as antimycin A which is believed essential to blocking the electron flow in the mitochondrial respiratory chain between cytochromes b and c<sub>1</sub>.<sup>9</sup> This fact suggested that the benzyl group and/or the absence of the C<sub>8</sub> methyl group decreased the cytotoxicity of **27** and that the selective toxicity against yeasts and filamentous fungi of UK-2A may stem from the feature of the nine-membered dilactone. The fact that (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A, UK-3A and (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A, which have no antifungal activities, show the similar cytotoxic activities to UK-2A suggested that it is quite probable to create a new antifungal agent with no cytotoxicity based upon the structure of UK-2A.

**Table 2.** Cytotoxic Activities

	ED <sub>50</sub> (μg/ml)								
	UK-2A	(7 <i>R</i> )-UK-2A	UK-3A	(7 <i>R</i> )-UK-3A	<b>27</b>	<b>28</b>	Antimycin A	Amphotericin B	Fluconazole
HEL <sup>a</sup>	74	57	38	51	>100	46	12	6.4	>100
P-388 <sup>b</sup>	37	80	14	74	8.5	30	<0.05	0.84	>100
EL-4 <sup>c</sup>	23	76	7.6	>100	3.4	30	<0.05	0.54	>100

<sup>a</sup> Human embryonic lung fibroblast. <sup>b</sup> Mouse leukemia cell. <sup>c</sup> Mouse lymphoma cell.

## Conclusion

In summary, we have developed a synthetic route to the naturally occurring form of UK-2A. Our route is highly stereoselective and applicable to the synthesis of their stereoisomers and analogs. In addition to the completion of total synthesis, this has allowed us to determine the assignment of the relative and absolute configurations in the nine-membered ring of UK-2A. We have synthesized another natural product, UK-3A, in the optical pure form as well. In order to define the selective cytotoxicities of UK-2A against yeasts and filamentous fungi, two types of UK-2A analogs were synthesized and subjected to the MIC evaluations and cytotoxic activity examinations along with UK-2A, (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A, UK-3A, (2*R*, 3*R*, 4*S*, 7*R*)-UK-3A, and antimycin A.

Table 1. *In Vitro* Antifungal Activities

Tested Organism	MIC ( $\mu\text{g/ml}$ )									
	UK-2A	(7 <i>F</i> )-UK-2A	UK-3A	(7 <i>F</i> )-UK-3A	27	28	Antimycin A	Amphotericin B	Fluconazole	
<i>Aspergillus fumigatus</i> KA-26	0.1	>100	>100	>100	0.1	>100	1.56	0.2	>100	
<i>Aspergillus fumigatus</i> TIMM 0069	0.1	>100	>100	>100	0.1	>100	1.56	0.2	>100	
<i>Aspergillus flavus</i> TIMM 0059	0.78	>100	>100	>100	3.13	>100	0.78	0.2	>100	
<i>Aspergillus niger</i> TIMM-2814	0.78	>100	>100	>100	3.13	>100	0.39	0.2	>100	
<i>Candida albicans</i> ATCC 10259	>100	>100	>100	>100	>100	>100	>100	0.1	>100	
<i>Candida tropicalis</i> M-6	>100	>100	>100	>100	>100	>100	>100	0.1	25	
<i>Cryptococcus neoformans</i> NI 7496	<0.05	>100	>100	>100	0.1	>100	<0.05	0.1	3.13	
<i>Cryptococcus neoformans</i> TIMM 0390	0.78	>100	>100	>100	0.78	>100	0.78	0.2	25	
<i>Trichophyton mentagrophytes</i> KD-04	0.1	>100	25	>100	1.56	>100	6.25	0.2	25	
<i>Trichophyton rubrum</i> KD-114	0.78	>100	12.5	>100	6.25	>100	6.25	0.2	12.5	

These results suggested that the basicity of the picolinic acid moiety in UK-2A was essential for the antifungal activities and that the feature of the nine-membered dilactone contributed to the selective cytotoxicities.

## Experimental Section

### Instrumentation

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at 270 MHz and 67.8 MHz, respectively, using a JEOL JNM-EX-270. Chemical shifts are reported in ppm ( $\delta$ ) relative to tetramethylsilane,  $\text{CDCl}_3$  with  $\text{CHCl}_3$  as the internal reference (7.24 ppm for  $^1\text{H}$  NMR and 77.0 ppm for  $^{13}\text{C}$  NMR), or  $\text{DMSO-}d_6$  with  $\text{DMSO-}d_5$  as the internal reference (2.49 ppm for  $^1\text{H}$  NMR and 39.5 ppm for  $^{13}\text{C}$  NMR), or  $\text{C}_6\text{D}_6$  with  $\text{C}_6\text{D}_5\text{H}$  as the internal reference (7.16 ppm for  $^1\text{H}$  NMR and 128.0 ppm for  $^{13}\text{C}$  NMR). Coupling constants are reported in hertz (Hz). Spectral splitting patterns are designed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Infrared spectra were recorded on a Shimadzu FTIR-4200. Gas-chromatography-mass-spectrometry was performed with a Shimadzu GCMS-QP1000EX. Microanalyses were performed with a Yanaco CHN CORDER MT-5. Mass spectra were measured on a JEOL HX-110A. Melting points were obtained from a Yanaco MP-500D and are uncorrected. Thin layer chromatography was performed on E. Merck and Co. precoated silica gel 60F254. Column chromatography was carried out with E. Merck and Co. silica gel 60 (70–230 mesh ASTM).

### Materials

Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use. Ether was dried over sodium at 25 °C for 3 days prior to use. DMF was dried over 13X molecular sieves at 25 °C for 3 days prior to use. *n*-Butyllithium and *t*-butyllithium were purchased from Kanto Kagaku Co., Inc. Dichloromethane and all secondary amines were dried over 4A molecular sieves prior to use. All other reagents were purchased from Nakarai Tesque or Wako Pure Chemical Industries and used without further purification unless otherwise stated. Air- and / or moisture- sensitive reactions were carried out under an atmosphere of argon.

**(4R)-4-Isopropyl-3-(3-phenylpropionyl)-oxazolidin-2-one (7)** To a stirred, cooled (-78 °C) solution of (*R*)-4-isopropylloxazolidin-2-one (11.0 g, 85.3 mmol) in THF (200 ml) was added *n*-butyllithium (1.58 M of hexane solution, 56.5 ml, 89.3 mmol) dropwise. The mixture was stirred for 20 minutes, treated with hydrocinnamoyl chloride (19 ml, 128 mmol) and stirred for 3 hours. After the mixture was quenched with ammonium chloride (saturated aq.), the resulting solution was gradually warmed to room temperature, and extracted with ether (3x). The combined organic layers were washed with 1 N NaOH aq. solution, and brine, and dried over  $\text{MgSO}_4$ . The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane-ether) to give (*R*)-4-isopropyl-3-(3-phenylpropionyl)-oxazolidin-2-one (**7**) (17.6 g, 78 %) as a colorless solid (m.p. 64.0 ~ 65.0 °C);  $[\alpha]_D^{25} +73.4^\circ$  (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 0.83 (d,  $J = 6.9$  Hz, 3H), 0.89 (d,  $J = 6.9$  Hz, 3H), 2.34 (m, 1H), 2.95-3.01 (m, 2H), 3.14-3.38 (m, 2H), 4.15-4.25 (m, 2H), 4.40 (m, 1H), 7.15-7.31 (m, 5H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 14.5 (q), 17.8 (q), 28.3 (d), 30.3 (t), 37.0 (t), 58.3 (d), 63.3 (t), 126.1 (d), 128.36 (d, 2C), 128.45 (d, 2C), 140.4 (s), 154.0 (s), 172.3 (s); IR ( $\text{CHCl}_3$ ) 1850, 1790, 1775, 1705, 1640, 1605, 1540, 1485, 1455, 1390, 1305, 1230, 1180,

1105, 1070, 1025, 1000, 970  $\text{cm}^{-1}$ ; FABMS:  $m/z$  262 (M+H)<sup>+</sup>; Anal. Calcd for  $\text{C}_{15}\text{H}_{19}\text{NO}_3$ : C: 68.96; H: 7.28; N: 5.36; Found: C: 68.83; H: 7.25; N: 5.27.

**(4R)-[(2R,3R,4S)-2-Benzyl-3-hydroxy-4-benzyloxypentanoyl]-4-isopropylloxazolidin-2-one**

**(8)** To a stirred, cooled (0 °C) solution of imide **7** (5.38 g, 20.6 mmol) in dichloromethane (60 ml) was added dibutylboron triflate (1.0 M of dichloromethane solution, 22.6 ml, 22.6 mmol) and triethylamine (4.0 ml, 28.7 mmol) dropwise successively. The mixture was stirred for 30 minutes at 0 °C, re-cooled to -78 °C, treated with a solution of aldehyde **6** (2.21 g, 13.5 mmol) in dichloromethane (11 ml), stirred at -78 °C for 1 hour, and at 0 °C for 2.5 hours. The mixture was then treated with a phosphate buffer solution (0.2 M, pH 7, 100 ml), re-cooled to -10 °C, treated with a solution of 30 %  $\text{H}_2\text{O}_2$  aq. (40 ml) in methanol (80 ml) dropwise over 30 minutes, and stirred at -10 °C for 1 hour. After quenching with  $\text{Na}_2\text{S}_2\text{O}_3$  (saturated aq.) dropwise, the resulting mixture was extracted with ethyl acetate (3x). The combined organic layers were washed with 5 %  $\text{NaHCO}_3$  aq. solution, and brine, and dried over  $\text{MgSO}_4$ . The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane-AcOEt) to give (4R)-[(2R,3R,4S)-2-benzyl-3-hydroxy-4-benzyloxypentanoyl]-4-isopropylloxazolidin-2-one (**8**) (2.58 g, 73 %) as a colorless crystalline solid (m.p. 125.0 ~ 127.0 °C);  $[\alpha]_D^{25} +30.42^\circ$  (c 0.802,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 0.17 (d, J = 6.6 Hz, 3H), 0.67 (d, J = 7.3 Hz, 3H), 1.32 (d, J = 5.9 Hz, 3H), 1.84 (m, 1H), 2.64 (d, J = 4.6 Hz, 1H), 2.96 (dd, J = 10.6, 13.2 Hz, 1H), 3.23 (dd, J = 5.3, 13.2 Hz, 1H), 3.49 (m, 2H), 3.82 (dd, J = 2.6, 8.9 Hz, 1H), 3.90 (m, 1H), 4.09 (dt, J = 8.6, 2.6 Hz, 1H), 4.30 (d, J = 11.9 Hz, 1H), 4.61 (d, J = 11.9 Hz, 1H), 4.81 (dt, J = 5.3, 10.2 Hz, 1H), 7.05-7.45 (m, 10H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 13.6 (q), 15.9 (q), 17.9 (q), 28.0 (d), 34.9 (t), 45.8 (d), 58.3 (d), 62.2 (t), 70.0 (t), 75.2 (d), 76.4 (d), 126.4 (d), 127.2 (d, 2C), 127.5 (d), 128.3 (d, 4C), 129.5 (d, 2C), 138.36 (s), 138.42 (s), 153.5 (s), 175.0 (s); IR (KBr) 3400, 2960, 1756, 1740, 1495, 1215, 1105, 740, 705  $\text{cm}^{-1}$ ; FABMS  $m/z$  426 (M+H)<sup>+</sup>, 408, 318, 189; HRFABMS Calcd. for  $\text{C}_{25}\text{H}_{32}\text{NO}_5$ : 426.2281; Found: 426.2290; Anal. Calcd for  $\text{C}_{25}\text{H}_{31}\text{NO}_5$ : C: 70.57; H: 7.34; N: 3.29; Found: C: 70.51; H: 7.28; N: 3.20.

**(2R,3R,4S)-2-Benzyl-3-(tert-butyldimethylsilyloxy)-4-benzyloxypentanoic acid (4)** To a stirred, cooled (0 °C) solution of aldol **8** (515 mg, 1.21 mmol) in THF- $\text{H}_2\text{O}$  (3 : 1, 24 ml) was added 30 %  $\text{H}_2\text{O}_2$  aq. (1.0 ml, 8.8 mmol) and 2N LiOH aq. (0.9 ml, 1.8 mmol) successively. After stirring for 13 hours at 25 °C, the mixture was quenched with 0.75 M  $\text{Na}_2\text{SO}_3$  aq. at 0 °C, stirred at 25 °C for 30 minutes, acidified with 5 % citric acid aq., and extracted with ethyl acetate (3x). The combined extracts were washed with brine, dried over  $\text{MgSO}_4$ , and concentrated. Silica gel column chromatography (ether-hexane) gave (R)-4-isopropylloxazolidin-2-one (125 mg, 81 % recovery) and (2R,3R,4S)-2-benzyl-3-hydroxy-4-benzyloxypentanoic acid (380 mg, 100 %) as a colorless crystalline solid (m.p. 133.0 ~ 134.0 °C);  $[\alpha]_D^{25} +54.4^\circ$  (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.25 (d, J = 5.9 Hz, 3H), 2.89 (br d, J = 10.6 Hz, 1H), 2.95 (br d, J = 9.7 Hz, 1H), 3.14 (m, 1H), 3.55 (m, 1H), 3.97 (t, J = 5.3 Hz, 1H), 4.38 (d, J = 11.6 Hz, 1H), 4.55 (d, J = 11.6 Hz, 1H), 7.10-7.38 (m, 10H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 14.3 (q), 33.8 (t), 49.4 (d), 70.6 (t), 73.0 (d), 75.3 (d), 126.4 (d), 127.8 (d, 3C), 128.37 (d, 2C), 128.42 (d, 2C), 128.9 (d, 2C), 137.9 (s), 138.8 (s), 179.2 (s); IR ( $\text{CHCl}_3$ ) 3420, 2950, 1720, 1640, 1500, 1280, 1100, 745, 700  $\text{cm}^{-1}$ ; FABMS  $m/z$  315 (M+H)<sup>+</sup>, 277, 241, 185; HRFABMS Calcd. for  $\text{C}_{19}\text{H}_{22}\text{O}_4$ : 315.1597; Found: 315.1595.

To a stirred solution of the above carboxylic acid (2.61 g, 9.78 mmol) in DMF (14.7 ml) was added imidazole (2.02 g, 29.6 mmol) and chloro *t*-butyldimethylsilane (2.24 g, 14.4 mmol) successively. After stirring for 6 hours at 25 °C, the mixture was quenched with  $\text{H}_2\text{O}$  (10 ml), stirred at 25 °C for 2.5 hours, and extracted



with ethyl acetate (3x). The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, and concentrated. Silica gel column chromatography (ether-hexane) gave (2*R*,3*R*,4*S*)-2-benzyl-3-(*tert*-butyldimethylsilyloxy)-4-benzyloxy-pentanoic acid (**4**) (3.72 g, 89 %) as a colorless oil;  $[\alpha]_D^{25} +13.27^\circ$  (c 1.01, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 0.03 (s, 3H), 0.08 (s, 3H), 0.92 (s, 9H), 1.21 (d, J = 5.9 Hz, 3H), 1.24 (br d, J = 8.9 Hz, 1H), 2.89 (br d, J = 9.6 Hz, 1H), 3.05 (m, 1H), 3.48 (m, 1H), 4.04 (t, J = 6.0 Hz, 1H), 4.38 (d, J = 11.9 Hz, 1H), 4.52 (d, J = 11.6 Hz, 1H), 7.06-7.34 (m, 10H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) -4.7 (q), -4.0 (q), 15.1 (d), 18.3 (s), 26.0 (q, 3C), 33.6 (t), 51.4 (d), 70.7 (t), 75.6 (d), 76.5 (d), 126.2 (d), 127.5 (d), 127.7 (d, 2C), 128.3 (d, 2C), 128.4 (d, 2C), 128.9 (d, 2C), 138.3 (s), 139.8 (s), 178.8 (s); IR (CHCl<sub>3</sub>): 2950, 1710, 1605, 1500, 1100, 780, 740, 695 cm<sup>-1</sup>; FABMS *m/z* 429 (M+H)<sup>+</sup>, 411, 371, 307, 154; HRFABMS Calcd. for C<sub>25</sub>H<sub>37</sub>O<sub>4</sub>Si: 429.2461; Found: 429.2455.

**Condensation of carboxylic acid 4 with *N*-Boc-L-Ser-OBn** To a stirred solution of carboxylic acid **4** (558 mg, 1.37 mmol) and *N*-Boc-L-Ser-OBn (487 mg, 1.65 mmol) in dichloromethane (5.0 ml) was added DMAP (16.4 mg, 0.13 mmol) and EDCI-HCl (323 mg, 1.68 mmol) successively. After stirring for 21 hours at 25 °C, the mixture was diluted with ether-hexane (1 : 2, 10 ml) and filtered through a short-pass silica gel column. The filtrate was concentrated and purified by silica gel column chromatography (ethyl acetate-hexane) to give the condensation product, (2*R*,3*R*,4*S*)-2-benzyl-4-benzyloxy-3-(*tert*-butyldimethylsilyloxy)-pentanoic acid (2*S*)-2-benzyloxy-carbonyl-2-*tert*-butoxycarbonylaminoethyl ester (**9**), (540 mg, 56 %) as a colorless oil;  $[\alpha]_D^{25} +29.93^\circ$  (c 0.608, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 0.09 (s, 3H), 0.10 (s, 3H), 0.93 (s, 9H), 1.12 (d, J = 6.3 Hz, 3H), 1.45 (s, 9H), 2.65-2.80 (m, 2H), 3.20 (d, J = 9.6 Hz, 1H), 3.31 (dq, J = 3.6, 6.3 Hz, 1H), 4.05 (m, 1H), 4.11-4.19 (m, 2H), 4.36 (m, 1H), 4.45 (s, 2H), 5.02 (d, J = 12.2 Hz, 1H), 5.10 (d, J = 12.2 Hz, 1H), 7.10 (d, J = 6.6 Hz, 2H), 7.08-7.37 (m, 13H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) -4.6 (q), -3.8 (q), 14.4 (q), 18.3 (s), 26.0 (q, 3C), 28.2 (q, 3C), 35.7 (t), 52.2 (d), 52.7 (d), 64.0 (t), 67.2 (t), 70.6 (t), 75.3 (d), 76.9 (d), 80.0 (s), 126.5 (d), 127.4 (d), 127.7 (d, 2C), 128.0 (d, 2C), 128.1 (d, 2C), 128.4 (d, 2C), 128.5 (d, 2C), 128.6 (d, 2C), 128.7 (d), 135.0 (s), 138.3 (s), 139.3 (s), 155.0 (s), 169.2 (s), 173.3 (d); IR (neat): 3420, 1740, 1720, 1605, 1500, 1455, 1390, 1370, 1360, 1255, 1160, 1105, 840 cm<sup>-1</sup>; FABMS *m/z* 706 (M+H)<sup>+</sup>; HRFABMS Calcd. for C<sub>40</sub>H<sub>55</sub>NO<sub>8</sub>Si: 706.3775; Found: 706.3770.

**Attempt to synthesize the nine-membered lactone 1 from hydroxycarboxylic acid 2** To a stirred solution of the condensation product **9** (170 mg, 0.24 mmol) in ethanol (4.0 ml) was added 10 % Pd(OH)<sub>2</sub>-C (34 mg). The resulting suspension was applied with H<sub>2</sub> gas (4.0 atm) and stirred vigorously at 25 °C for 14 hours. Then the mixture was filtered through Celite column and concentrated to give the crude seco acid, (2*R*,3*R*,4*S*)-2-benzyl-3-(*tert*-butyldimethylsilyloxy)-4-hydroxypentanoic acid (2*S*)-2-*tert*-butoxycarbonylamino-2-carboxyethyl ester (**2**), (126 mg, 100 %).

To a stirred, cooled (0 °C) solution of the above seco acid **2** (84 mg, 0.119 mmol) in dichloromethane (150 ml) was added triethylamine (0.25 ml, 1.79 mmol), 4-dimethylaminopyridine (400 mg, 3.27 mmol) and 2,4,6-trichlorobenzoyl chloride (0.23 ml, 1.47 mmol) successively. After stirring for 14 hours at 25 °C, the mixture was washed with 5 % citric acid aq., saturated NaHCO<sub>3</sub> aq., and brine successively. The resulting solution was dried over MgSO<sub>4</sub>, and concentrated. Silica gel column chromatography (ethyl acetate-hexane) gave (3*R*,4*R*,5*S*)-3-benzyl-4-(*tert*-butyldimethylsilyloxy)-5-methyldihydrofuran-2-one (**11**) (26.0 mg, 68 %) as a colorless crystalline solid (m.p. 58.0 ~ 59.0 °C);  $[\alpha]_D^{25} -27.8^\circ$  (c 1.04, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) -0.05 (s, 3H), -0.02 (s, 3H), 0.85 (s, 9H), 1.25 (d, J = 6.6 Hz, 3H), 2.90 (dd, J = 6.3, 12.5 Hz, 1H),

2.97–3.12 (m, 2H), 3.80 (dd,  $J = 5.0, 5.9$  Hz, 1H), 4.23 (dq,  $J = 5.0, 6.6$  Hz, 1H), 7.21–7.34 (m, 5H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) -4.6 (q), -4.5 (q), 17.7 (s), 18.7 (q), 25.5 (q, 3C), 33.8 (t), 51.4 (d), 77.3 (d), 81.8 (d), 126.8 (d), 128.6 (d, 2C), 129.2 (d, 2C), 137.7 (s), 176.3 (s); IR (KBr) : 1755, 1645(br), 1500, 1470, 1455, 1385, 1360, 1315, 1255, 1190, 1115, 1090, 1045, 1020, 955, 880, 845  $\text{cm}^{-1}$ ; FABMS  $m/z$  321 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{18}\text{H}_{29}\text{O}_3\text{Si}$ : 321.1886; Found: 321.1889.

**(S)-2-(4-Methoxybenzyloxy)-propional (15)** To a stirred solution of ethyl (*S*)-lactate (15.0 g, 0.109 mol) in dichloromethane (300 ml) was added 4-methoxybenzyloxy-trichloromethylimidate (60 g, 0.213 mol) and 10-camphorsulfonic acid (2.5 g, 11.0 mmol) successively. After stirring for 16 hours at 25 °C, the mixture was diluted with hexanes (600 ml) and filtered to remove precipitates. The filtrate was concentrated and purified by silica gel column chromatography (ethyl acetate-hexane) to give ethyl (*S*)-2-(4-methoxybenzyloxy)-propionate (26.0 g, 86 %) as a colorless oil;  $[\alpha]_D^{27} -69.4^\circ$  (c 1.04,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.30 (t,  $J = 6.9$  Hz, 3H), 1.41 (d,  $J = 6.9$  Hz, 3H), 3.80 (s, 3H), 4.03 (q,  $J = 6.9$  Hz, 1H), 4.22 (q,  $J = 7.3$  Hz, 2H), 4.39 (d,  $J = 11.2$  Hz, 1H), 4.62 (d,  $J = 11.2$  Hz, 1H), 6.88 (d,  $J = 8.6$  Hz, 2H), 7.29 (d,  $J = 8.6$  Hz, 2H); FABMS  $m/z$  239 (M+H)<sup>+</sup>.

To a stirred, cooled (-78 °C) solution of the above ethyl (*S*)-2-(4-methoxybenzyloxy)-propionate (21.0 g, 88.0 mmol) in THF (200 ml) was added diisobutylaluminum hydride (0.93 M of hexane solution, 100 ml, 93 mmol) dropwise. After stirring for 2 hours at -78 °C, the mixture was quenched with saturated sodium potassium tartrate aq. (500ml), stirred at 25 °C for 1 hour and extracted with ethyl acetate (3x). The combined extracts were washed with brine, dried over  $\text{MgSO}_4$  and concentrated. Silica gel column chromatography (ethyl acetate-hexane) gave (*S*)-2-(4-methoxybenzyloxy)-propional (**15**) (14.3 g, 83 %) as a colorless oil;  $[\alpha]_D^{23} -40.87^\circ$  (c 1.03,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.31 (t,  $J = 6.9$  Hz, 3H), 3.81 (s, 3H), 3.87 (dq,  $J = 1.7, 6.9$  Hz, 1H), 4.53 (d,  $J = 11.2$  Hz, 1H), 4.58 (d,  $J = 11.2$  Hz, 1H), 6.89 (d,  $J = 8.6$  Hz, 2H), 7.29 (d,  $J = 8.6$  Hz, 2H), 9.64 (d,  $J = 1.7$  Hz, 1H); FABMS  $m/z$  194 (M+H)<sup>+</sup>.

**(4R)-[(2R,3R,4S)-2-Benzyl-3-hydroxy-4-(4-methoxybenzyloxy)-pentanoyl]-4-isopropyl-oxazolidin-2-one (16)** This was prepared from imide **7** (22.0 g, 84.1 mmol) and (*S*)-2-(4-methoxybenzyloxy)propional (**15**) (10.9 g, 56.2 mmol) by the procedure for aldol **8**. (*4R*)-[(2*R*,3*R*,4*S*)-2-benzyl-3-hydroxy-4-methoxybenzyloxy-pentanoyl]-4-isopropyl-oxazolidin-2-one (**16**) (21.0 g, 82 %) was obtained as a colorless crystalline solid (m.p. 94 ~ 95 °C);  $[\alpha]_D^{25} +19.7^\circ$  (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 0.22 (d,  $J = 6.9$  Hz, 3H), 0.70 (d,  $J = 6.9$  Hz, 3H), 1.29 (d,  $J = 5.9$  Hz, 3H), 1.89 (dq,  $J = 3.3, 6.9, 6.9$  Hz, 1H), 2.61 (d,  $J = 4.3$  Hz, 1H), 2.96 (dd,  $J = 10.2, 13.5$  Hz, 1H), 3.18 (dd,  $J = 5.3, 13.5$  Hz, 1H), 3.47 (dq,  $J = 5.9, 5.9$  Hz, 1H), 3.69 (t,  $J = 8.6$  Hz, 1H), 3.78 (s, 3H), 3.81–3.92 (m, 2H), 4.15 (dt,  $J = 8.6, 3.3$  Hz, 1H), 4.24 (d,  $J = 11.2$  Hz, 1H), 4.53 (d,  $J = 11.2$  Hz, 1H), 4.79 (ddd,  $J = 5.3, 5.3, 10.2$  Hz, 1H), 6.87 (d,  $J = 8.6$  Hz, 2H), 7.10–7.29 (m, 7H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 13.6 (q), 15.9 (q), 17.9 (q), 28.0 (d), 34.7 (t), 45.6 (d), 55.2 (q), 58.3 (d), 62.2 (t), 69.7 (t), 75.1 (d), 75.9 (d), 113.6 (d, 2C), 126.4 (d), 128.2 (d, 2C), 128.8 (d, 2C), 129.4 (d, 2C), 130.4 (d), 138.4 (s), 153.4 (s), 159.0 (s), 175.1 (s); IR ( $\text{CHCl}_3$ ) 3040, 1765, 1745, 1690, 1670, 1615, 1585, 1515, 1495, 1460, 1380, 1300, 1250, 1220, 1200, 1180, 1140, 1100, 1035, 980, 940  $\text{cm}^{-1}$ ; FABMS  $m/z$  456 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{26}\text{H}_{34}\text{NO}_6$ : 456.2386; Found: 456.2394.

**(2R,3R,4S)-2-Benzyl-3-hydroxy-4-(4-methoxybenzyloxy)-pentanoic acid benzyl ester (17)**

To a stirred, cooled (0 °C) solution of aldol **16** (4.44 g, 9.76 mmol) in THF-H<sub>2</sub>O (3 : 1, 160 ml) was added 30 % H<sub>2</sub>O<sub>2</sub> aq. (8.0 ml, 71.0 mmol) and 2 N LiOH aq. (7.2 ml, 14.4 mmol) successively. After stirring for 12 hours at 25 °C, the mixture was quenched with 0.75 M Na<sub>2</sub>SO<sub>3</sub> aq. at 0 °C, stirred at 25 °C for 30 minutes, acidified with 5 % citric acid aq., and extracted with ethyl acetate (3x). The combined extracts were washed with brine, dried over MgSO<sub>4</sub>, and concentrated. Silica gel column chromatography (ether-hexane) gave (*R*)-4-isopropylloxazolidin-2-one (1.07 g, 85 % recovery) and (2*R*,3*R*,4*S*)-2-benzyl-3-hydroxy-4-(4-methoxybenzyloxy)-pentanoic acid (3.09 g, 92 %) as a colorless crystalline solid (m.p. 96.0 ~ 99.0 °C); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +46.15° (c 1.04, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 1.22 (d, J = 6.3 Hz, 3H), 2.88 (br d, J = 9.6 Hz, 1H), 2.94 (dd, J = 2.6, 9.6 Hz, 1H), 3.12 (m, 1H), 3.53 (m, 1H), 3.78 (s, 3H), 3.95 (t, J = 5.3 Hz, 1H), 4.31 (d, J = 10.9 Hz, 1H), 4.48 (d, J = 10.9 Hz, 1H), 6.85 (d, J = 8.6 Hz, 2H), 7.10-7.28 (m, 7H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) 14.4 (q), 33.7 (t), 49.3 (d), 55.2 (q), 70.2 (t), 73.0 (d), 74.9 (d), 113.8 (d, 2C), 126.4 (d), 128.4 (d, 2C), 128.9 (d, 2C), 129.5 (d, 2C), 129.9 (s), 138.9 (s), 159.2 (s), 179.2 (s); IR (KBr) 3550, 3200, 1728, 1685, 1515, 1242, 1100, 820, 705 cm<sup>-1</sup>; FABMS *m/z* 345 (M+H)<sup>+</sup>, 277, 241, 185, 149; HRFABMS Calcd. for C<sub>20</sub>H<sub>25</sub>O<sub>5</sub>: 345.1702; Found: 345.1701; Anal. Calcd for C<sub>20</sub>H<sub>24</sub>O<sub>5</sub>: C: 69.75; H: 7.02; Found: C: 69.61; H: 6.90.

To a stirred solution of the above carboxylic acid (8.04 g, 23.4 mmol) and benzyl alcohol (4.9 ml, 47.3 mmol) in THF (80.0 ml) was added triphenylphosphine (12.3 g, 46.9 mmol) and DIAD (9.2 ml, 46.5 mmol) successively. After stirring for 40 minutes at 25 °C, the mixture was concentrated and purified by silica gel column chromatography (ethyl acetate-hexane) to give (2*R*,3*R*,4*S*)-2-benzyl-3-hydroxy-4-(4-methoxybenzyloxy)-pentanoic acid benzyl ester (**17**) (8.99 g, 89 %) as a colorless oil; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +48.71° (c 1.01, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 1.20 (d, J = 6.3 Hz, 3H), 2.72 (d, J = 3.0 Hz, 1H), 2.85-2.97 (m, 2H), 3.17 (m, 1H), 3.42 (dq, J = 5.0, 6.3 Hz, 1H), 3.78 (s, 3H), 3.96 (m, 1H), 4.29 (d, J = 11.2 Hz, 1H), 4.44 (d, J = 11.2 Hz, 1H), 4.88 (s, 2H), 6.86 (d, J = 8.6 Hz, 2H), 7.02-7.05 (m, 2H), 7.10-7.13 (m, 2H), 7.18-7.28 (m, 8H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) 14.2 (q), 34.4 (t), 49.8 (d), 55.2 (d), 66.2 (t), 70.2 (t), 73.3 (d), 74.9 (d), 113.8 (d, 2C), 126.3 (d), 128.1 (d), 128.2 (d, 2C), 128.3 (d, 2C), 128.4 (d, 2C), 128.9 (d, 2C), 129.4 (d, 2C), 130.2 (s), 135.3 (s), 139.0 (s), 159.2 (s), 173.9 (s); IR (neat) 3480, 1735, 1615, 1585, 1515, 1500, 1460, 1385, 1250, 1175, 1095, 1035, 980 cm<sup>-1</sup>; FABMS *m/z* 435 (M+H)<sup>+</sup>; HRFABMS Calcd. for C<sub>27</sub>H<sub>31</sub>O<sub>5</sub>: 435.2171; Found: 435.2143.

**(2R,3R,4S)-2-Benzyl-3-(tert-butyldimethylsilyloxy)-4-hydroxypentanoic acid benzyl ester (14)**

To a stirred solution of benzyl ester **17** (8.7 g, 20.0 mmol) in DMF (20 ml) was added imidazole (5.6 g, 81.5 mmol) and chloro *t*-butyldimethylsilylamine (6.1 g, 40.1 mmol) successively. After stirring for 17 hours at 60 °C, the mixture was quenched with H<sub>2</sub>O (60 ml), stirred at 25 °C for 30 minutes and extracted with ethyl acetate. The extract was washed with brine, dried over MgSO<sub>4</sub>, and concentrated. Silica gel column chromatography (ethyl acetate-hexane) gave (2*R*,3*R*,4*S*)-2-benzyl-3-(*tert*-butyldimethylsilyloxy)-4-(4-methoxybenzyloxy)-pentanoic acid benzyl ester (10.9 g, 99 %) as a colorless oil; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +32.02° (c 1.01, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 0.08 (s, 3H), 0.09 (s, 3H), 0.93 (s, 9H), 1.14 (d, J = 6.3 Hz, 3H), 2.77-2.88 (m, 2H), 3.18 (m, 1H), 3.29 (dq, J = 3.0, 6.3 Hz, 1H), 3.78 (s, 3H), 4.07 (dd, J = 3.0, 6.6 Hz, 1H), 4.30 (d, J = 11.2 Hz, 1H), 4.35 (d, J = 11.2 Hz, 1H), 6.83 (d, J = 7.9 Hz, 2H), 7.04 (m, 2H), 7.11 (m, 2H), 7.16-7.27 (m, 8H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) -4.7 (q), -3.8 (q), 14.3 (d), 18.4 (s), 26.1 (q, 3C), 35.4 (t), 52.4 (d), 55.2 (d), 66.0 (t), 70.1 (t), 75.5 (d), 76.4 (d), 113.5 (d, 2C), 126.1 (d), 128.0 (d), 128.2 (d, 2C), 128.3 (d, 4C), 128.9 (d,

2C), 129.2 (d, 2C), 130.6 (d), 135.6 (s), 139.7 (s), 158.9 (s), 173.6 (s); IR (neat) 1735, 1615, 1585, 1515, 1500, 1460, 1385, 1360, 1300, 1250, 1175, 1145, 1100(sh), 1040, 940, 840  $\text{cm}^{-1}$ ; FABMS  $m/z$  549 (M+H)<sup>+</sup>; HRFABMS Calcd. for C<sub>33</sub>H<sub>45</sub>O<sub>5</sub>Si: 549.3036; Found: 549.3061.

To a stirred solution of the above silyl ether (200 mg, 0.37 mmol) in dichloromethane-H<sub>2</sub>O (10 : 1, 6.0 ml) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (124 mg, 0.55 mmol). After stirring for 30 minutes at 25 °C, the mixture was poured into saturated NaHCO<sub>3</sub> aq., extracted with ether, washed with saturated NaHCO<sub>3</sub> aq. repeatedly (until the aqueous layer was not stained with the residue of DDQ and/or its reduced form), brine, dried over MgSO<sub>4</sub> and concentrated. The crude alcohol, (2*R*,3*R*,4*S*)-2-benzyl-3-(*tert*-butyldimethylsilyloxy)-4-hydroxypentanoic acid benzyl ester (**14**), (210 mg, 100 %) was used for the next reaction without further purification; FABMS  $m/z$  429 (M+H)<sup>+</sup>.

**Condensation of alcohol 14 with *N*-Boc-L-Ser(OBn), preparation of (2*R*,3*R*,4*S*)-2-Benzyl-4-[(2*S*)-3-benzyloxy-2-*tert*-butoxycarbonylamino propionyloxy]-3-(*tert*-butyldimethyl-silyloxy)-pentanoic acid benzyl ester [(*S*)-18]**

To a stirred, cooled (0 °C) solution of crude alcohol **14** (210 mg, 0.37 mmol) and *N*-Boc-L-Ser(OBn) (324 mg, 1.1 mmol) in dichloromethane (2.5 ml) was added DMAP (13.4 mg, 0.11 mmol) and EDCI (170 mg, 1.1 mmol) successively. After stirring for 2 hours at 10 °C, the mixture was diluted with ether-hexane (1 : 2, 10 ml) and filtered through a short-pass silica gel column. The filtrate was concentrated and purified by silica gel column chromatography (ethyl acetate-hexane) to give the condensation product, (2*R*,3*R*,4*S*)-2-benzyl-4-[(2*S*)-3-benzyloxy-2-*tert*-butoxycarbonylamino propionyloxy]-3-(*tert*-butyldimethylsilyloxy)-pentanoic acid benzyl ester [(*S*)-**18**] (238 mg, 91 % from **17**) as a colorless oil;  $[\alpha]_D^{25} +16.88^\circ$  (c 0.80, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 0.03 (s, 3H), 0.06 (s, 3H), 0.92 (s, 9H), 1.25 (d, J = 6.3 Hz, 3H), 1.43 (s, 9H), 2.83 (m, 2H), 3.10 (m, 1H), 3.62 (dd, J = 3.0, 9.6 Hz, 1H), 3.84 (dd, J = 3.3, 9.6 Hz, 1H), 4.03 (dd, J = 3.3, 6.0 Hz, 1H), 4.42 (br s, 3H), 4.95 (m, 3H), 5.19 (d, J = 3.6 Hz, 1H), 6.90-7.50 (m, 15H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>) -4.6 (q), -4.1 (q), 14.6 (d), 18.3 (s), 25.9 (q, 3C), 28.3 (q, 3C), 34.8 (t), 51.8 (d), 54.1 (d), 66.5 (t), 70.0 (t), 73.1 (t), 73.9 (d), 75.1 (d), 79.9 (s), 126.3 (d), 127.6 (d, 3C), 127.8 (d), 128.1 (d), 128.3 (d, 2C), 128.4 (d, 5C), 128.9 (d, 2C), 135.3 (s), 137.5 (s), 139.2 (s), 155.2 (s), 169.8 (s), 173.0 (s); IR (neat) 2950, 1735, 1500, 1160, 780, 742, 700  $\text{cm}^{-1}$ ; FABMS  $m/z$  706 (M+H)<sup>+</sup>; HRFABMS Calcd. for C<sub>40</sub>H<sub>55</sub>NO<sub>8</sub>Si: 706.3775; Found: 706.3766.

**Nine-membered dilactone, [(3*S*,7*R*,8*R*,9*S*)-7-benzyl-8-(*tert*-butyldimethylsilyloxy)-9-methyl-2,6-dioxo-[1,5]dioxonane-3-yl]-carbamic acid *tert*-butyl ester, [(*S*)-19]**

To a stirred solution of the condensation product (*S*)-**18** (1.12 g, 1.59 mmol) in ethanol (25 ml) was added 10 % Pd(OH)<sub>2</sub> (160 mg). The resulting suspension was applied with H<sub>2</sub> gas (4.0 atm) and stirred vigorously at 25 °C for 14 hours. Then the mixture was filtered through Celite pad and concentrated to give the crude seco acid, (2*R*,3*R*,4*S*)-2-benzyl-4-[(2*S*)-2-*tert*-butoxycarbonylamino-3-hydroxypropionyloxy]-3-(*tert*-butyldimethyl-silyloxy)-pentanoic acid, [(*S*)-**12**] (834 mg, 100 %).

To a stirred solution of the above seco acid (*S*)-**12** (834 mg, 1.59 mmol) in dichloromethane (1.5 l) was added triphenylphosphine (2.5 g, 9.53 mmol) and DIAD (1.9 ml, 9.59 mmol) successively. After stirring for 24 hours at 25 °C, the mixture was concentrated and purified by silica gel column chromatography (ethyl acetate-hexane) to give the nine-membered dilactone, [(3*S*,7*R*,8*R*,9*S*)-7-benzyl-8-(*tert*-butyldimethylsilyloxy)-9-methyl-2,6-dioxo-[1,5]dioxonane-3-yl]-carbamic acid *tert*-butyl ester, [(*S*)-**19**] (698 mg, 87 %) as a colorless crystalline solid (m.p. 117 ~ 120 °C);  $[\alpha]_D^{25} +70.68^\circ$  (c 1.01, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 0.16 (s,

3H), 0.21 (s, 3H), 0.96 (s, 9H), 1.39 (d,  $J = 6.6$  Hz, 3H), 1.42 (s, 9H), 2.68 (m, 1H), 2.78 (t,  $J = 2.5$  Hz, 1H), 3.10 (m, 1H), 3.41 (dd,  $J = 5.9, 10.2$  Hz, 1H), 3.77 (t,  $J = 8.9$  Hz, 1H), 4.73 (m, 2H), 5.20 (m, 2H), 7.12 (d,  $J = 6.9$  Hz, 1H), 7.17–7.28 (m, 4H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) -3.1 (q, 2C), 18.2 (q), 19.0 (s), 25.9 (q, 3C), 28.2 (q, 3C), 35.5 (t), 51.8 (d), 55.4 (d), 66.1 (t), 77.3 (d, 2C), 80.4 (s), 126.5 (d), 128.5 (d, 2C), 128.8 (d, 2C), 138.6 (s), 154.7 (s), 170.7 (s), 173.4 (s); IR (KBr) 3400, 1750, 1725, 1640, 1500, 1455, 1370, 1330, 1200, 1165, 1095, 1060, 840  $\text{cm}^{-1}$ ; FABMS  $m/z$  508 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{26}\text{H}_{42}\text{NO}_7\text{Si}$ : 508.2731; Found: 508.2704.

**Isobutyric acid (3*S*,6*S*,7*R*,8*R*)-8-benzyl-3-*tert*-butoxycarbonylamino-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester [(*S*)-10]** Dilactone (*S*)-19 (777 mg, 1.53 mmol) was treated with (HF-pyridine complex)-pyridine-THF (5 : 3 : 8, 8.0 ml) at room temperature and stirred until (*S*)-19 disappeared (ca. 3 days). The mixture was diluted with ethyl acetate, dropped into a stirred saturated  $\text{NaHCO}_3$  aq., and extracted with ethyl acetate (2x). The combined extracts were washed with brine, dried over  $\text{MgSO}_4$  and concentrated. Silica gel column chromatography (ethyl acetate-hexane) gave [(3*S*,7*R*,8*R*,9*S*)-7-benzyl-8-hydroxy-9-methyl-2,6-dioxo-[1,5]dioxonane-3-yl]-carbamic acid *tert*-butyl ester, (626 mg, 100 %) as a colorless crystalline solid (m.p. 83 ~ 88 °C);  $[\alpha]_D^{25} +89.5^\circ$  (c 1.01,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.42 (s, 9H), 1.42–1.46 (m, 3H), 2.70 (m, 1H), 2.93 (br d,  $J = 13.2$  Hz, 1H), 3.02 (br d,  $J = 7.9$  Hz, 1H), 3.23 (dd,  $J = 2.6, 13.2$  Hz, 1H), 3.42 (dd,  $J = 6.6, 9.9$  Hz, 1H), 3.67 (ddd,  $J = 7.3, 9.2, 9.2$  Hz, 1H), 4.72–4.83 (m, 2H), 5.18 (br t,  $J = 9.2$  Hz, 1H), 5.30 (br d,  $J = 8.3$  Hz, 1H), 7.15–7.28 (m, 5H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 18.3 (q), 28.2 (q, 3C), 35.0 (t), 51.4 (d), 54.0 (d), 65.4 (t), 76.4 (d), 76.7 (d), 80.6 (s), 126.4 (d), 128.5 (d, 2C), 128.8 (d, 2C), 138.6 (s), 154.9 (s), 170.9 (s), 172.9 (s); IR (KBr) 3400, 1755, 1725, 1695, 1640, 1525, 1455, 1365, 1335, 1200, 1165, 1045, 890  $\text{cm}^{-1}$ ; FABMS  $m/z$  394 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{20}\text{H}_{28}\text{NO}_7$ : 394.1866; Found: 394.1875.

To a stirred, cooled (0 °C) solution of the above alcohol (413 mg, 1.05 mmol) in pyridine (5.0 ml) was added isobutyryl chloride (0.44 ml, 4.20 mmol). After stirring for 5.5 hours at 0 °C, the mixture was quenched with  $\text{H}_2\text{O}$  (5 ml), stirred at 25 °C for 30 minutes and extracted with ether. The extract was washed with saturated  $\text{NaHCO}_3$  aq., brine, dried over  $\text{MgSO}_4$ , and concentrated. Silica gel column chromatography (ethyl acetate-hexane) gave isobutyric acid (3*S*,6*S*,7*R*,8*R*)-8-benzyl-3-*tert*-butoxycarbonylamino-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester [(*S*)-10] (456 mg, 94 %) as a colorless crystalline solid (m.p. 148 ~ 150 °C);  $[\alpha]_D^{25} +79.0^\circ$  (c 1.01,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.229 (d,  $J = 6.9$  Hz, 3H), 1.234 (d,  $J = 6.9$  Hz, 3H), 1.29 (d,  $J = 6.3$  Hz, 3H), 1.42 (s, 9H), 2.61 (septet,  $J = 6.9$  Hz, 1H), 2.68 (d,  $J = 10.9$  Hz, 1H), 2.85–3.02 (m, 2H), 3.44 (m, 1H), 4.79 (m, 1H), 4.91 (dq,  $J = 9.9, 6.3$  Hz, 1H), 5.18 (t,  $J = 9.9$  Hz, 1H), 5.14–5.21 (m, 1H), 7.16–7.28 (m, 5H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 17.8 (q), 18.9 (q, 2C), 28.2 (q, 3C), 34.0 (d), 34.5 (t), 51.4 (d), 51.8 (d), 65.7 (t), 74.4 (d), 75.0 (d), 80.5 (s), 126.6 (d), 128.5 (d, 2C), 128.7 (d, 2C), 137.9 (s), 154.6 (s), 170.8 (s), 171.7 (s), 175.6 (s); IR (KBr) 3360, 1770, 1740, 1720, 1695, 1515, 1450, 1390, 1365, 1350, 1330, 1240, 1150(br), 1070, 1020, 890  $\text{cm}^{-1}$ ; FABMS  $m/z$  464 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{24}\text{H}_{34}\text{NO}_8$ : 464.2284; Found: 464.2278.

**(2*R*,3*R*,4*S*)-2-Benzyl-4-[(2*R*)-3-benzyloxy-2-*tert*-butoxycarbonylamino-propionyloxy]-3-(*tert*-butyldimethyl-silanyloxy)-pentanoic acid benzyl ester [(*R*)-18]** This was prepared from crude alcohol **14** (320 mg, 0.55 mmol) and *N*-Boc-D-Ser(OBn) (586 mg, 1.65 mmol) by the procedure for (*S*)-18. The reaction temperature was -10 °C. (2*R*,3*R*,4*S*)-2-Benzyl-4-[(2*R*)-3-benzyloxy-2-*tert*-butoxycarbonylamino-

propionyloxy]-3-(*tert*-butyldimethylsilyloxy)-pentanoic acid benzyl ester [(*R*)-**18**] (382 mg, 89 % from **17**) was obtained as a colorless oil;  $[\alpha]_D^{25} +10.4^\circ$  (c 1.07,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ ) 0.08 (s, 6H), 0.92 (s, 9H), 1.17 (d,  $J = 6.3$  Hz, 3H), 1.45 (s, 9H), 2.70–2.85 (m, 2H), 3.12 (m, 1H), 3.66 (dd,  $J = 3.0, 9.2$  Hz, 1H), 3.85 (dd,  $J = 3.3, 9.2$  Hz, 1H), 4.05 (dd,  $J = 2.6, 6.9$  Hz, 1H), 4.38–4.47 (m, 2H), 4.52 (d,  $J = 12.2$  Hz, 1H), 4.97 (s, 2H), 5.01 (m, 1H), 5.43 (d,  $J = 8.6$  Hz, 1H), 7.03–7.13 (m, 4H), 7.16–7.36 (m, 11H);  $^{13}\text{C NMR}$  (67.8 MHz,  $\text{CDCl}_3$ ) -4.5 (q), -4.0 (q), 13.9 (d), 18.3 (s), 25.9 (q, 3C), 28.3 (q, 3C), 35.3 (t), 52.4 (d), 54.0 (d), 66.5 (t), 70.1 (t), 73.3 (t), 73.5 (d), 74.9 (d), 79.9 (s), 126.3 (d), 127.6 (d, 2C), 127.8 (d), 128.1 (d), 128.3 (d, 3C), 128.4 (d, 5C), 128.8 (d, 2C), 135.3 (s), 137.4 (s), 139.0 (s), 155.3 (s), 169.7 (s), 172.8 (s); IR (KBr) 2950, 1740, 1500, 1460, 1110, 835, 780, 745, 700  $\text{cm}^{-1}$ ; FABMS  $m/z$  706 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{40}\text{H}_{55}\text{NO}_8\text{Si}$ : 706.3775; Found: 706.3790.

**[(3*R*,7*R*,8*R*,9*S*)-7-Benzyl-8-(*tert*-butyldimethylsilyloxy)-9-methyl-2,6-dioxo-[1,5]dioxonane-3-yl]-carbamic acid *tert*-butyl ester [(*R*)-**19**] This was prepared from (*R*)-**18** (662 mg, 0.94 mmol) by the procedure for (*S*)-**19**. [(3*R*,7*R*,8*R*,9*S*)-7-Benzyl-8-(*tert*-butyldimethylsilyloxy)-9-methyl-2,6-dioxo-[1,5]dioxonane-3-yl]-carbamic acid *tert*-butyl ester [(*R*)-**19**] (382 mg, 80 %) was obtained as a colorless crystalline solid (m.p. 71 ~ 75 °C);  $[\alpha]_D^{25} +85.38^\circ$  (c 0.814,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ ) 0.18 (s, 3H), 0.22 (s, 3H), 0.96 (s, 9H), 1.39 (d,  $J = 6.3$  Hz, 3H), 1.43 (s, 9H), 2.69–2.82 (m, 2H), 3.15 (m, 1H), 3.78 (t,  $J = 8.6$  Hz, 1H), 4.10 (m, 1H), 4.53–4.60 (m, 2H), 4.74 (dq,  $J = 8.6, 6.6$  Hz, 1H), 5.61 (br d,  $J = 8.6$  Hz, 1H), 7.11 (d,  $J = 6.6$  Hz, 2H), 7.16–7.28 (m, 3H);  $^{13}\text{C NMR}$  (67.8 MHz,  $\text{CDCl}_3$ ) -3.1 (q), -2.9 (q), 18.2 (q), 19.0 (s), 25.9 (q, 3C), 28.2 (q, 3C), 35.6 (t), 55.3 (d), 56.0 (d), 66.9 (t), 77.2 (d, 2C), 80.2 (s), 126.5 (d), 128.5 (d, 2C), 128.7 (d, 2C), 138.4 (s), 154.9 (s), 168.3 (s), 175.2 (s); IR (KBr) 3430, 1760(br), 1740, 1720, 1640, 1495, 1370, 1295, 1270, 1215, 1065, 1095, 1065, 840  $\text{cm}^{-1}$ ; FABMS  $m/z$  508 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{26}\text{H}_{42}\text{NO}_7\text{Si}$ : 508.2731; Found: 508.2723.**

**Isobutyric acid (3*R*,6*S*,7*R*,8*R*)-8-benzyl-3-*tert*-butoxycarbonylamino-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester [(*R*)-**10**] This was prepared from dilactone (*R*)-**19** (285 mg, 0.562 mmol) through the corresponding alcohol by the procedure for isobutyryl ester (*S*)-**10**.**

The intermediate alcohol, [(3*R*,7*R*,8*R*,9*S*)-7-benzyl-8-hydroxy-9-methyl-2,6-dioxo-[1,5]dioxonane-3-yl]-carbamic acid *tert*-butyl ester (224 mg, 100 %) as a colorless crystalline solid (m.p. 80 ~ 90 °C);  $[\alpha]_D^{25} +102.4^\circ$  (c 0.706,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ ) 1.43 (s, 9H), 1.43–1.48 (m, 3H), 2.64 (m, 1H), 2.76 (dt,  $J = 3.3, 10.4$  Hz, 1H), 2.95 (m, 1H), 3.28 (br d,  $J = 12.9$  Hz, 1H), 3.69 (dd,  $J = 9.2, 16.5$  Hz, 1H), 4.14 (m, 1H), 4.60 (m, 2H), 4.80 (dq,  $J = 9.2, 6.3$  Hz, 1H), 5.66 (br d,  $J = 8.6$  Hz, 1H), 7.18–7.31 (m, 5H);  $^{13}\text{C NMR}$  (67.8 MHz,  $\text{CDCl}_3$ ) 18.2 (q), 28.2 (q, 3C), 35.0 (t), 54.0 (d), 55.9 (d), 66.4 (t), 76.5 (d), 76.8 (d), 80.5 (s), 126.4 (d), 128.4 (d, 2C), 128.7 (d, 2C), 138.5 (s), 155.1 (s), 168.4 (s), 175.0 (s); IR (KBr) 3400(br), 1755, 1740(sh), 1695, 1640, 1500, 1455, 1370, 1300, 1250, 1220, 1165, 1150, 1065  $\text{cm}^{-1}$ ; FABMS  $m/z$  394 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{20}\text{H}_{28}\text{NO}_7$ : 394.1866; Found: 394.1852.

Isobutyric acid (3*R*,6*S*,7*R*,8*R*)-8-benzyl-3-*tert*-butoxycarbonylamino-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester [(*R*)-**10**] (238 mg, 90 %) was obtained as a colorless crystalline solid (m.p. 147 ~ 148 °C);  $[\alpha]_D^{25} +94.2^\circ$  (c 0.50,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ ) 1.24 (d,  $J = 6.9$  Hz, 6H), 1.30 (d,  $J = 6.3$  Hz, 3H), 1.44 (s, 9H), 2.63 (septet,  $J = 6.9$  Hz, 1H), 2.71 (br d,  $J = 8.9$  Hz, 1H), 2.91 (d,  $J = 10.5$  Hz, 1H), 2.98 (d,  $J = 10.5$  Hz, 1H), 4.15 (m, 1H), 4.59–4.66 (m, 2H), 4.93 (dq,  $J = 9.9, 6.3$  Hz, 1H), 5.20 (t,  $J = 8.9$  Hz, 1H), 5.65 (br d,  $J = 8.9$  Hz, 1H), 7.10 (d,  $J = 7.3$  Hz, 2H), 7.16–7.27 (m, 3H); ;  $^{13}\text{C NMR}$  (67.8 MHz,  $\text{CDCl}_3$ ) 17.6 (q),

18.87 (q), 18.91 (q), 28.2 (q, 3C), 34.0 (d), 34.5 (t), 51.8 (d), 55.9 (d), 66.8 (t), 74.4 (d), 74.7 (d), 80.3 (s), 126.7 (d), 128.5 (d, 2C), 128.6 (d, 2C), 137.6 (s), 155.0 (s), 168.3 (s), 173.7 (s), 175.7 (s); IR (KBr) 3400(br), 3400, 1745, 1715, 1640, 1505, 1455, 1370, 1345, 1300, 1250, 1145(sh)  $\text{cm}^{-1}$ ; FABMS  $m/z$  464 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{24}\text{H}_{34}\text{NO}_8$ : 464.2284; Found: 464.2288.

**3-Methoxymethoxypyridine (21)** A modification of the reported procedure was used which gave much better yield. To a stirred, cooled (-15 °C) solution of 3-hydroxypyridine (**20**) (70.0 g, 0.74 mol) in THF-DMF (3:8, 550 ml) was added *t*-BuOK (91.0 g, 0.81 mol) in one shot. After stirring at -15 °C for 25 minutes, the mixture was treated with chloromethylmethyl ether (62.2 g, 0.77 mol) dropwise over one hour. The mixture was stirred for another one hour, then gradually warmed to 15 °C. The resulting dark brown tar mixture was concentrated to remove THF, poured into brine, extracted with ethyl acetate, and washed with  $\text{H}_2\text{O}$  (4x) and brine successively. The all aqueous layers were combined, extracted with ethyl acetate, and washed with  $\text{H}_2\text{O}$  (4x) and brine successively. The combined extracts were concentrated, filtered through  $\text{Na}_2\text{SO}_4$ -silica gel short-pass column (80% EtOAc / hexane), and concentrated. The residue was distilled under reduced pressure to give 3-methoxymethoxypyridine (**21**) (73.0 g, 71.3 %) as a colorless oil (b.p. 66.0 – 69.0 °C/4 mmHg);  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 3.49 (s, 3H), 5.20 (s, 2H), 7.22 (dd,  $J = 5.0, 8.6$  Hz, 1H), 7.37 (m, 1H), 8.27 (d,  $J = 4.6$  Hz, 1H), 8.41 (d,  $J = 3.0$  Hz, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 56.1 (q), 94.6 (t), 122.9 (d), 123.8 (d), 139.6 (d), 143.2 (d), 153.5 (s); IR (neat) 2950, 1578, 1480, 1430, 1155, 990, 805, 710  $\text{cm}^{-1}$ ; EIMS :  $m/z$  139 (M<sup>+</sup>), 108, 78.

**4-Bromo-3-methoxymethoxypyridine (22)** To a stirred, cooled (-78 °C) solution of 3-methoxymethoxypyridine (**21**) (8.92 g, 64.1 mmol) in  $\text{Et}_2\text{O}$  (300 ml) was added *t*-BuLi (1.20 M of pentane solution, 53.0 ml, 63.6 mmol) dropwise. After stirring at -78 °C for 30 minutes, the mixture was treated with a solution of 1,2-dibromo-1,1,2,2-tetrafluoroethane (20.0 g, 77.0 mmol) in THF (15 ml) dropwise. The mixture was stirred for another 20 minutes and gradually warmed to -20 °C. The resulting mixture was poured into  $\text{H}_2\text{O}$ , extracted with  $\text{CHCl}_3$  (3x), dried over  $\text{MgSO}_4$  and concentrated. The crude residue was diluted with ethyl acetate-hexane (2 : 1, 50 ml), filtered through  $\text{MgSO}_4$  column in order to remove  $\text{H}_2\text{O}$ , and concentrated to give crude 4-bromo-3-methoxymethoxy-pyridine (**22**) (14.3 g). This was used immediately for the next reaction because of its instability.;  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 3.55 (s, 3H), 5.30 (s, 2H), 7.51 (d,  $J = 5.0$  Hz, 1 H), 8.11 (d,  $J = 5.0$  Hz, 1H), 8.45 (s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 56.6 (q), 95.6 (t), 122.9 (s), 128.2 (d), 138.5 (d), 143.9 (d), 151.0 (s).

**4-Methoxy-3-methoxymethoxypyridine (23)** To stirred methanol (100 ml) was added a piece of metallic sodium (10.0 g, 0.43 mol). After the metallic sodium disappeared, the above 4-bromo-3-methoxymethoxypyridine (**22**) (14.3 g) in methanol (5 ml) was added carefully into the refluxing NaOMe solution and the mixture was refluxed for 40 minutes. The resulting mixture was cooled to room temperature, diluted with  $\text{Et}_2\text{O}$ , quenched with dry-ice, and poured into  $\text{H}_2\text{O}$ , and extracted with  $\text{CHCl}_3$  (3x). All extracts were combined, dried over  $\text{MgSO}_4$  and concentrated. Silica gel column chromatography (ethyl acetate-hexane → ethyl acetate) gave 4-methoxy-3-methoxymethoxypyridine (**23**) (8.82 g, 81.3 %) as a clear oil;  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 3.54 (s, 3H), 3.92 (s, 3H), 5.23 (s, 2H), 6.83 (d,  $J = 5.6$  Hz, 1H), 8.23 (d,  $J = 5.6$  Hz, 1H), 8.37 (s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 55.6 (q), 56.4 (q), 96.0 (t), 106.8 (d), 139.0 (d), 143.2 (s), 145.5 (d), 156.0 (s); IR (neat) 2950, 1585, 1515, 1300, 1025, 820  $\text{cm}^{-1}$ ; EIMS :  $m/z$  169 (M<sup>+</sup>), 140, 124.

**3-Hydroxy-4-methoxypyridine-2-carboxylic acid (5)** To a stirred, cooled (-78 °C) solution of 4-methoxy-3-methoxymethoxypyridine (**23**) (21.6 g, 128 mmol) in THF (620 ml) was added *t*-BuLi (1.63 M of pentane solution, 82.0 ml, 134 mmol) dropwise. After stirring at -78 °C for 30 minutes, the mixture was treated with excess dry-ice in one shot and stirred for another 20 minutes at -78 °C. Then the mixture was allowed to warm to room temperature and concentrated. The residue was triturated with Et<sub>2</sub>O-hexane (2 : 1, 500 ml), and pale yellow powder was collected and washed with plenty of Et<sub>2</sub>O-hexane (2 : 1). The powder was dried in vacuo to give crude lithium 4-methoxy-3-methoxymethoxypyridine-2-carboxylate (27.5 g). Then this was treated with 1 N HCl (124 ml, 124 mmol) for 3 days. The white precipitate was collected, washed with plenty of EtOH-Et<sub>2</sub>O (1 : 2), and dried in vacuo in the presence of P<sub>2</sub>O<sub>5</sub> to give 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) (20.9 g, 97 %) as a white solid (m.p. 226.0 ~ 227.0 °C); <sup>1</sup>H NMR (270 MHz, DMSO-*d*<sub>6</sub>) 4.03 (s, 3H), 7.39 (d, J = 6.4 Hz, 1H), 8.03 (d, J = 6.4 Hz, 1H), 17.04 (br s, 1H); <sup>13</sup>C NMR (67.8 MHz, DMSO-*d*<sub>6</sub>) 57.4 (q), 109.2 (d), 126.6 (s), 132.4 (s), 152.5 (s), 162.1 (s), 164.2 (s); IR (KBr) 3450, 2700, 1665, 1600, 1530, 1305, 840, 785 cm<sup>-1</sup>; MS (FAB) : m/z 170 (M+H)<sup>+</sup>, 149, 93; Anal. Calcd for C<sub>7</sub>H<sub>7</sub>NO<sub>4</sub>: C: 49.71; H: 4.17; N: 8.28; Found: C: 49.50; H: 4.16; N: 8.26.

**Isobutyric acid (3*S*,6*S*,7*R*,8*R*)-3-amino-8-benzyl-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester [(*S*)-**1**]** To a stirred solution of (*S*)-**10** (447 mg, 0.965 mmol) in dichloromethane (4.0 ml) was added trifluoroacetic acid (4.0 ml) dropwise. After stirring at 25 °C for 2 hours, the mixture was concentrated, diluted with saturated NaHCO<sub>3</sub> aq., extracted with ethyl acetate (2x). The combined extracts were washed with brine, dried over MgSO<sub>4</sub> and concentrated to give crude primary amine, isobutyric acid (3*S*,6*S*,7*R*,8*R*)-3-amino-8-benzyl-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester, [(*S*)-**1**] (366 mg, 100 %). This was used for the next reaction without further purification.

**(2*R*, 3*R*, 4*S*, 7*S*)-UK-2A** To a stirred solution of primary amine (*S*)-**1** (366 mg, 0.965 mmol) and 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) (245 mg, 1.45 mmol) in dimethylformamide (3.0 ml) was added HOBt (235 mg, 1.74 mmol), EDCI-HCl (333 mg, 1.74 mmol) and *N*-methylmorpholine (0.74 ml, 6.76 mmol) successively. After stirring for 10 hours at 25 °C, the mixture was poured into H<sub>2</sub>O and extracted with ethyl acetate (3x). The combined extracts were washed with H<sub>2</sub>O (2x) and brine, dried over MgSO<sub>4</sub> and concentrated. Silica gel column chromatography (ethyl acetate-hexane) gave (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A (253 mg, 51 % from (*S*)-**10**) as a colorless crystalline solid (m.p. 201.0 ~ 203.0 °C); [α]<sup>23</sup><sub>D</sub> +89.29° (c 1.008, CHCl<sub>3</sub>); <sup>1</sup>H NMR (270 MHz, C<sub>6</sub>D<sub>6</sub>) 0.99 (d, J = 7.0 Hz, 3H), 1.01 (d, J = 7.0 Hz, 3H), 1.12 (d, J = 6.6 Hz, 3H), 2.26 (septet, J = 7.0 Hz, 1H), 2.77 (dd, J = 3.3, 13.2 Hz, 1H), 2.89 (dt, J = 3.0, 9.9 Hz, 1H), 3.07 (br s, 1H), 3.12 (s, 3H), 3.16 (dd, J = 11.6, 13.2 Hz, 1H), 5.01 (dq, J = 9.6, 6.3 Hz, 1H), 5.06 (m, 1H), 5.19 (m, 1H), 5.43 (t, J = 9.9 Hz, 1H), 6.06 (d, J = 5.3 Hz, 1H), 7.05 (m, 1H), 7.12 (m, 4H), 7.71 (d, J = 5.3 Hz, 1H), 8.78 (br d, J = 7.3 Hz, 1H), 12.44 (s, 1H); <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) 1.24 (d, J = 6.9 Hz, 6H), 1.33 (d, J = 6.9 Hz, 3H), 2.63 (septet, J = 6.9 Hz, 1H), 2.71 (br d, J = 10.6 Hz, 1H), 2.94 (dd, J = 13.2, 11.9 Hz, 1H), 3.01 (t, J = 11.5 Hz, 1H), 3.61 (m, 1H), 3.94 (s, 3H), 4.98 (dq, J = 9.9, 6.3 Hz, 1H), 5.17 (m, 1H), 5.22 (t, J = 9.9 Hz, 1H), 5.35 (m, 1H), 6.88 (d, J = 5.3 Hz, 1H), 7.13 (d, J = 6.9 Hz, 2H), 7.22 (m, 1H), 7.26 (m, 2H), 7.99 (d, J = 5.3 Hz, 1H), 8.59 (d, J = 8.2 Hz, 1H), 11.78 (s, 1H); <sup>13</sup>C NMR (67.8 MHz, C<sub>6</sub>D<sub>6</sub>) 17.74 (q), 18.86 (q, 2C), 34.13 (d), 35.08 (t), 50.50 (d), 52.39 (d), 55.19 (q), 65.27 (t), 74.70 (d), 75.28 (d), 109.85 (d), 126.94 (d), 128.81 (d, 2C), 129.20 (d, 2C), 130.49 (s), 138.40 (s), 140.40 (d), 149.96 (s), 155.78 (s), 169.40



(s), 169.98 (s), 171.70 (s), 175.01 (s);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 17.86 (q), 18.99 (q, 2C), 34.11 (d), 34.56 (t), 49.94 (d), 51.95 (d), 56.14 (q), 65.21 (t), 74.77 (d), 74.97 (d), 109.70 (d), 126.72 (d), 128.62 (d, 2C), 128.77 (d, 2C), 129.92 (s), 137.86 (s), 140.73 (d), 148.82 (s), 155.42 (s), 168.96 (s), 169.77 (s), 171.77 (s), 175.65 (s); IR (KBr) 3400, 1745, 1650, 1540, 1140, 700  $\text{cm}^{-1}$ ; FABMS  $m/z$  515 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{26}\text{H}_{31}\text{N}_2\text{O}_9$ : 515.2030; Found: 515.2045; Anal. Calcd for  $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}_9$ : C: 60.69; H: 5.88; N: 5.44; Found: C: 60.57; H: 5.91; N: 5.57.

**Isobutyric acid (3*R*,6*S*,7*R*,8*R*)-3-amino-8-benzyl-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester [(*R*)-1]** This was prepared from (*R*)-10 (230 mg, 0.496 mmol) by the procedure for (*S*)-1. Crude primary amine, isobutyric acid (3*S*,6*S*,7*R*,8*R*)-3-amino-8-benzyl-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester, [(*R*)-1] (209 mg, 100 %) was obtained as a colorless oil and was used for the next reaction without further purification.

**(2*R*, 3*R*, 4*S*, 7*R*)-UK-2A** This was prepared from (*R*)-1 (209 mg, 0.496 mmol) and 3-hydroxy-4-methoxy-pyridine-2-carboxylic acid (**5**) (126 mg, 0.745 mmol) by the procedure for (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A. (2*R*, 3*R*, 4*S*, 7*R*)-UK-2A (156 mg, 61 % from (*R*)-10) was obtained as a colorless crystalline solid (m.p. 233 ~ 235 °C);  $[\alpha]_D^{23} +90.77^\circ$  (c 0.802,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{C}_6\text{D}_6$ ) 0.89 (d,  $J = 6.9$  Hz, 3H), 0.91 (d,  $J = 6.9$  Hz, 3H), 0.99 (d,  $J = 5.9$  Hz, 3H), 2.16 (septet,  $J = 6.9$  Hz, 1H), 2.68 (dd,  $J = 2.6, 13.2$  Hz, 1H), 2.89 (dt,  $J = 10.7, 3.0$  Hz, 1H), 3.05 (s, 3H), 3.07 (m, 1H), 3.43 (m, 1H), 4.21 (br d,  $J = 12.2$  Hz, 1H), 4.80 (t,  $J = 7.6$  Hz, 1H), 4.98 (dq,  $J = 9.6, 5.9$  Hz, 1H), 5.31 (t,  $J = 9.6$  Hz, 1H), 5.95 (d,  $J = 5.3$  Hz, 1H), 7.03 (m, 1H), 7.06 (m, 2H), 7.10 (m, 2H), 7.79 (d,  $J = 5.3$  Hz, 1H), 9.36 (br d,  $J = 8.3$  Hz, 1H), 12.61 (s, 1H);  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.24 (d,  $J = 6.9$  Hz, 3H), 1.25 (d,  $J = 6.9$  Hz, 3H), 1.30 (d,  $J = 6.3$  Hz, 3H), 2.64 (septet,  $J = 6.9$  Hz, 1H), 2.71 (d,  $J = 10.6$  Hz, 1H), 2.99 (dd,  $J = 13.5, 11.5$  Hz, 1H), 3.04 (t,  $J = 11.9$  Hz, 1H), 3.96 (s, 3H), 4.26 (m, 1H), 4.82 (br d,  $J = 11.6$  Hz, 1H), 4.97 (m, 1H), 5.00 (t,  $J = 6.9$  Hz, 1H), 5.25 (t,  $J = 9.6$  Hz, 1H), 6.92 (d,  $J = 5.3$  Hz, 1H), 7.13-7.32 (m, 5H), 8.15 (d,  $J = 5.3$  Hz, 1H), 8.92 (br d,  $J = 8.9$  Hz, 1H), 11.9 (s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{C}_6\text{D}_6$ ) 17.51 (q), 18.73 (q), 18.86 (q), 34.06 (d), 35.05 (t), 52.39 (d), 54.56 (d), 55.14 (q), 65.90 (t), 75.05 (d), 75.14 (d), 109.89 (d), 126.99 (d), 128.81 (d, 2C), 129.22 (d, 2C), 130.64 (s), 138.37 (s), 140.92 (d), 150.05 (s), 155.69 (s), 167.93 (s), 169.55 (s), 173.66 (s), 174.86 (s);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 17.68 (q), 18.94 (q), 19.00 (q), 34.13 (d), 34.47 (t), 50.02 (d), 53.91 (d), 56.08 (q), 66.15 (t), 74.57 (d), 75.04 (d), 109.58 (d), 126.74 (d), 128.61 (d, 2C), 128.72 (d, 2C), 130.03 (s), 137.73 (s), 141.17 (d), 148.77 (s), 155.27 (s), 167.49 (s), 168.79 (s), 173.44 (s), 175.74 (s); IR (KBr) 3350, 1750, 1650, 1580, 1530, 1325, 1145, 1065, 1030, 850  $\text{cm}^{-1}$ ; FABMS  $m/z$  515 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{26}\text{H}_{31}\text{N}_2\text{O}_9$ : 515.2029; Found: 515.2020.

**(3*R*,4*R*,5*S*)-3-Benzyl-4-(*tert*-butyldimethylsilyloxy)-5-methyldihydrofuran-2-one (11)** A solution of hydroxy ester **14** (164 mg, 0.312 mmol) in dichloromethane (6.0 ml) was left at 25 °C for 3 days. Then the mixture was concentrated and purified by silica gel column chromatography (hexane-ethyl acetate) to give (3*R*,4*R*,5*S*)-3-benzyl-4-(*tert*-butyldimethylsilyloxy)-5-methyldihydrofuran-2-one (**11**) (80 mg, 80 %) as a colorless crystalline solid.

**(3*R*,4*R*,5*S*)-3-Benzyl-4-hydroxy-5-methyldihydrofuran-2-one (24)**  $\gamma$ -Lactone **11** (66.6 mg, 0.216 mmol) was treated with (HF-pyridine complex)-pyridine-THF (5 : 3 : 8, 2.0 ml) at room temperature and stirred

until  $\gamma$ -lactone **11** disappeared (ca. 1 hour). The mixture was diluted with ethyl acetate, dropped into a stirred saturated  $\text{NaHCO}_3$  aq., and extracted with ethyl acetate (2x). The combined extracts were washed with brine, dried over  $\text{MgSO}_4$  and concentrated. Silica gel column chromatography (ethyl acetate-hexane) gave (3*R*,4*R*,5*S*)-3-benzyl-4-hydroxy-5-methyl-dihydrofuran-2-one (**24**) (37.8 mg, 90 %) as a colorless crystalline solid (m.p. 51.0 ~ 52.0 °C);  $[\alpha]^{25}_{\text{D}} -91.0^\circ$  (c 0.50,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (270 MHz,  $\text{C}_6\text{D}_6$ ) 0.96 (d,  $J = 6.3$  Hz, 3H), 2.12 (d,  $J = 5.3$  Hz, 1H), 2.54 (ddd,  $J = 5.9, 6.9, 9.2$  Hz, 1H), 2.78 (dd,  $J = 6.9, 14.2$  Hz, 1H), 3.03 (dd,  $J = 5.6, 14.2$  Hz, 1H), 3.43 (m, 1H), 3.83 (quintet,  $J = 6.6$  Hz, 1H), 6.98-7.15 (m, 5H);  $^{13}\text{C NMR}$  (67.8 MHz,  $\text{C}_6\text{D}_6$ ) 17.7 (q), 33.7 (t), 50.2 (d), 77.5 (d), 79.9 (d), 127.0 (d), 128.9 (d, 2C), 129.6 (d, 2C), 138.2 (s), 175.3 (s); IR (KBr) 3450, 2950, 1760, 1640, 1500, 1180, 1060, 760, 700  $\text{cm}^{-1}$ ; FABMS  $m/z$  207 ( $\text{M}+\text{H}$ )<sup>+</sup>, 185, 149, 93; HRFABMS Calcd. for  $\text{C}_{12}\text{H}_{15}\text{O}_3$ ; 207.1021; Found: 207.1033.

**(2*S*,3*R*,4*R*)-Isobutyric acid 4-benzyl-2-methyl-5-oxo-tetrahydrofuran-3-yl ester (25)** This was prepared from  $\gamma$ -Lactone **25** (100 mg, 0.49 mmol) by the procedure for isobutyryl ester (*S*)-**10**. (2*S*,3*R*,4*R*)-Isobutyric acid 4-benzyl-2-methyl-5-oxo-tetrahydrofuran-3-yl ester (**25**) (115 mg, 85 %) was obtained as a colorless oil;  $[\alpha]^{25}_{\text{D}} -16.2^\circ$  (c 1.01,  $\text{CHCl}_3$ )<sup>33</sup>;  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ ) 1.01 (d,  $J = 6.9$  Hz, 3H), 1.06 (d,  $J = 6.9$  Hz, 3H), 1.16 (d,  $J = 6.6$  Hz, 3H), 2.37 (septet,  $J = 6.9$  Hz, 1H), 2.98 (dd,  $J = 12.9, 7.9$  Hz, 1H), 3.06 (m, 1H), 3.26 (dd,  $J = 12.9, 4.3$  Hz, 1H), 4.29 (qd,  $J = 6.6, 5.0$  Hz, 1H), 4.95 (dd,  $J = 5.9, 5.0$  Hz, 1H), 7.18-7.35 (m, 5H);  $^{13}\text{C NMR}$  (67.8 MHz,  $\text{CDCl}_3$ ) 18.5 (q), 18.6 (q), 18.8 (q), 33.5 (d), 34.6 (t), 48.3 (d), 77.4 (d), 79.3 (d), 127.0 (d), 128.7 (d, 2C), 129.2 (d, 2C), 137.1 (s), 175.2 (s), 176.2 (s); IR (neat) 2990, 1785, 1740, 1605, 1500, 1455, 1030, 755, 705  $\text{cm}^{-1}$ ; FABMS  $m/z$  277 ( $\text{M}+\text{H}$ )<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{16}\text{H}_{21}\text{O}_4$ ; 277.1439; Found: 277.1451.

**(2*S*)-3-Hydroxy-2-[(3-hydroxy-4-methoxypyridine-2-carbonyl)-amino]-propanoic acid methyl ester [(*S*)-**26**]** This was prepared from L-serine methyl ester hydrochloride (1.1 g, 7.08 mmol) and 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) (1.0 g, 5.9 mmol) by the procedure for (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A. Silica gel column chromatography (ethyl acetate-hexane) followed by re-crystallization (ethyl acetate-hexane) gave (2*S*)-3-hydroxy-2-[(3-hydroxy-4-methoxypyridine-2-carbonyl)-amino]-propanoic acid methyl ester [(*S*)-**26**] (450 mg, 28 %) as a colorless crystalline solid (m.p. 124.0 ~ 125.0 °C);  $[\alpha]^{27}_{\text{D}} +29.00^\circ$  (c 0.443,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ ) 1.02 (br s, 1H), 3.84 (s, 3H), 3.95 (s, 3H), 4.11 (m, 2H), 4.81 (dt,  $J = 7.9, 4.0$  Hz, 1H), 6.87 (d,  $J = 5.3$  Hz, 1H), 7.98 (d,  $J = 5.3$  Hz, 1H), 8.75 (br d,  $J = 7.6$  Hz, 1H), 11.95 (s, 1H);  $^{13}\text{C NMR}$  (67.8 MHz,  $\text{CDCl}_3$ ) 52.9 (q), 54.2 (d), 56.1 (q), 63.1 (t), 109.5 (d), 130.2 (s), 140.7 (d), 148.7 (s), 155.4 (s), 169.2 (s), 170.3 (s); IR (KBr) 3400, 3150, 1750, 1630, 1550, 1300, 1220, 820, 785, 740  $\text{cm}^{-1}$ ; FABMS  $m/z$  271 ( $\text{M}+\text{H}$ )<sup>+</sup>, 241, 185, 149; Anal. Calcd for  $\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_6$ : C: 48.89; H: 5.22; N: 10.37; Found: C: 48.68; H: 5.32; N: 10.46.

**(2*R*)-3-Hydroxy-2-[(3-hydroxy-4-methoxypyridine-2-carbonyl)-amino]-propanoic acid methyl ester [(*R*)-**26**]** This was prepared from D-serine methyl ester hydrochloride (1.1 g, 7.08 mmol) and 3-hydroxy-4-methoxypyridine-2-carboxylic acid (**5**) (1.0 g, 5.9 mmol) by the procedure for (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A. Silica gel column chromatography (ethyl acetate-hexane) followed by re-crystallization (ethyl acetate-hexane) gave (2*R*)-3-hydroxy-2-[(3-hydroxy-4-methoxypyridine-2-carbonyl)-amino]-propanoic acid methyl ester [(*R*)-**26**] (534 mg, 33 %) as a colorless crystalline solid (m.p. 123.0 ~ 125.0 °C);  $[\alpha]^{27}_{\text{D}} -29.72^\circ$  (c 1.006,  $\text{CHCl}_3$ );

$^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 2.94 (br s, 1H), 3.83 (s, 3H), 3.98 (s, 3H), 4.11 (m, 2H), 4.81 (dt,  $J = 7.6, 3.6$  Hz, 1H), 6.86 (d,  $J = 5.3$  Hz, 1H), 7.97 (d,  $J = 5.3$  Hz, 1H), 8.74 (br d,  $J = 7.9$  Hz, 1H), 11.95 (s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 52.9 (q), 54.3 (d), 56.1 (q), 63.0 (t), 109.5 (d), 130.1 (s), 140.6 (d), 148.6 (s), 155.3 (s), 169.1 (s), 170.3 (s); IR (KBr) 3400, 1750, 1540, 1520, 1300, 810, 790  $\text{cm}^{-1}$ ; FABMS  $m/z$  515 ( $\text{M}+\text{H}^+$ ); HRFABMS Calcd. for  $\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_6$ : 271.0930; Found: 271.0925; Anal. Calcd for  $\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_6$ : C: 48.89; H: 5.22; N: 10.37; Found: C: 48.87; H: 5.16; N: 10.35.

**(2R, 3R, 4S, 7S)-UK-3A** This was prepared from (*S*)-1 (85 mg, 0.234 mmol) and 3-hydroxypicolinic acid (49 mg, 0.351 mmol) by the procedure for (2R, 3R, 4S, 7S)-UK-2A. (2R, 3R, 4S, 7S)-UK-3A (80 mg, 71 % from (*S*)-10) was obtained as a colorless crystalline solid (m.p. 179.0 ~ 182.0  $^{\circ}\text{C}$ );  $[\alpha]^{25}_{\text{D}} +81.70^{\circ}$  (c 0.306,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{C}_6\text{D}_6$ ) 0.95 (d,  $J = 6.9$  Hz, 3H), 0.97 (d,  $J = 6.9$  Hz, 3H), 1.07 (d,  $J = 6.3$  Hz, 1H), 2.16 (septet,  $J = 6.9$  Hz, 1H), 2.72 (dd,  $J = 3.3, 13.5$  Hz, 1H), 2.89 (ddd,  $J = 3.3, 10.2, 11.2$  Hz, 1H), 2.99 (br s, 1H), 3.11 (dd,  $J = 11.5, 13.2$  Hz, 1H), 4.95 (dq,  $J = 9.9, 6.3$  Hz, 1H), 5.01 (m, 1H), 5.13 (m, 1H), 5.38 (t,  $J = 9.9$  Hz, 1H), 6.51 (dd,  $J = 4.3, 8.6$  Hz, 1H), 6.88 (dd,  $J = 1.3, 8.6$  Hz, 1H), 7.00 (m, 1H), 7.08 (m, 4H), 7.65 (dd,  $J = 1.3, 4.3$  Hz, 1H), 8.55 (br d,  $J = 7.6$  Hz, 1H), 12.14 (s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{C}_6\text{D}_6$ ) 17.71 (q), 18.82 (q), 18.89 (q), 34.11 (d), 35.08 (t), 50.43 (d), 52.39 (d), 65.16 (t), 74.78 (d), 75.24 (d), 126.01 (d), 126.96 (d), 128.83 (d, 2C), 128.97 (d), 129.20 (d, 2C), 131.11 (s), 138.39 (s), 139.63 (s), 158.46 (s), 168.90 (s), 169.94 (s), 171.72 (s), 175.03 (s); IR (KBr) 3450, 2950, 1745, 1650, 1535, 1450, 1140, 700  $\text{cm}^{-1}$ ; FABMS  $m/z$  485 ( $\text{M}+\text{H}^+$ ), 461, 425, 277, 185; HRFABMS Calcd. for  $\text{C}_{25}\text{H}_{29}\text{N}_2\text{O}_8$ : 485.1941; Found: 485.1924; Anal. Calcd for  $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_8$ : C: 61.98; H: 5.82; N: 5.78; Found: C: 62.09; H: 5.99; N: 5.85.

**(2R, 3R, 4S, 7R)-UK-3A** This was prepared from (*R*)-1 (67.2 mg, 0.17 mmol) and 3-hydroxypicolinic acid (36 mg, 0.26 mmol) by the procedure for (2R, 3R, 4S, 7S)-UK-2A. (2R, 3R, 4S, 7R)-UK-3A (72.3 mg, 90 % from (*R*)-10) was obtained as a colorless crystalline solid (m.p. 193.0 ~ 196.0  $^{\circ}\text{C}$ );  $[\alpha]^{25}_{\text{D}} +87.5^{\circ}$  (c 0.304,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{C}_6\text{D}_6$ ) 0.87 (d,  $J = 6.9$  Hz, 3H), 0.90 (d,  $J = 6.9$  Hz, 3H), 1.00 (d,  $J = 6.3$  Hz, 1H), 2.16 (septet,  $J = 6.9$  Hz, 1H), 2.67 (dd,  $J = 3.0, 12.2$  Hz, 1H), 2.89 (dt,  $J = 3.0, 12.2$  Hz, 1H), 3.05 (t,  $J = 12.2$  Hz, 1H), 3.43 (m, 1H), 4.18 (br d,  $J = 12.2$  Hz, 1H), 4.79 (t,  $J = 7.6$  Hz, 1H), 4.98 (m, 1H), 5.30 (t,  $J = 9.9$  Hz, 1H), 6.48 (dd,  $J = 4.3, 8.6$  Hz, 1H), 6.88 (dd,  $J = 0.7, 8.6$  Hz, 1H), 6.97-7.21 (m, 4H), 7.82 (d,  $J = 4.3$  Hz, 1H), 9.24 (br d,  $J = 8.2$  Hz, 1H), 12.4 (s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{C}_6\text{D}_6$ ) 17.54 (q), 18.71 (q), 18.84 (q), 34.06 (d), 35.03 (t), 52.37 (d), 54.52 (d), 65.83 (t), 75.10 (d, 2C), 125.92 (d), 127.01 (d), 128.81 (d, 2C), 128.97 (d), 129.20 (d, 2C), 1331.27 (s), 138.33 (s), 140.17 (d), 158.60 (s), 167.86 (s), 169.06 (s), 173.68 (s), 174.85 (s); IR (KBr) 3450 (br), 1750, 1650, 1600, 1530, 1450, 1300, 1250, 1220, 1185, 1145, 1065, 1030  $\text{cm}^{-1}$ ; FABMS  $m/z$  485 ( $\text{M}+\text{H}^+$ ), 461, 369, 277, 185; HRFABMS Calcd. for  $\text{C}_{25}\text{H}_{29}\text{N}_2\text{O}_8$ : 485.1941; Found: 485.1958; Anal. Calcd for  $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_8$ : C: 61.98; H: 5.82; N: 5.78; Found: C: 61.89; H: 5.87; N: 5.70.

**Isobutyric acid (3S,6S,7R,8R)-8-benzyl-3-(3-formylamino-2-hydroxybenzoylamino)-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester (27)** This was prepared from (7S)-1 (228 mg, 0.628 mmol) and 3-formamidosalicylic acid (171 mg, 0.942 mmol) by the procedure for (2R, 3R, 4S, 7S)-UK-2A. Isobutyric acid (3S,6S,7R,8R)-8-benzyl-3-(3-formylamino-2-hydroxybenzoylamino)-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester (27) (144 mg, 44 % from (*S*)-10) was obtained as a colorless crystalline solid (m.p.

123.0 ~ 125.0 °C);  $[\alpha]^{25}_{\text{D}} +97.6^\circ$  (c 0.53,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.25 (d,  $J = 6.9$  Hz, 6H), 1.33 (d,  $J = 6.3$  Hz, 3H), 2.64 (m, 1H), 2.73 (m, 1H), 2.97 (m, 2H), 3.63 (br s, 1H), 5.01 (m, 1H), 5.23 (m, 1H), 5.30 (m, 1H), 5.47 (br t,  $J = 9.2$  Hz, 1H), 6.86 (t,  $J = 7.9$  Hz, 1H), 7.08–7.38 (m, 6H), 8.09 (br s, 1H), 8.50 (br s, 1H), 8.52 (d,  $J = 9.2$  Hz, 1H), 12.60 (s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 17.7 (q), 18.9 (q, 2C), 34.1 (d), 34.5 (t), 50.7 (d), 52.0 (d), 65.6 (t), 74.8 (d), 75.1 (d), 112.4 (s), 118.8 (d), 120.2 (d), 124.7 (d), 126.7 (d), 127.3 (s), 128.6 (d, 2C), 128.7 (d, 2C), 137.7 (s), 150.5 (s), 159.2 (d), 169.5 (s), 170.2 (s), 171.8 (s), 175.7 (s); IR (KBr) 3400, 2970, 1752, 1682, 1640, 1535, 1140, 750, 700  $\text{cm}^{-1}$ ; FABMS  $m/z$  527 (M+H)<sup>+</sup>, 461, 369, 277, 185; HRFABMS Calcd. for  $\text{C}_{27}\text{H}_{31}\text{N}_2\text{O}_9$ : 527.2030; Found: 527.2035.

**Isobutyric acid (3*S*,6*S*,7*R*,8*R*)-8-benzyl-3-(2-hydroxy-3-methoxybenzoylamino)-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester (28)** This was prepared from (7*S*)-1 (138 mg, 0.38 mmol) and 3-methoxysalicylic acid (96 mg, 0.57 mmol) by the procedure for (2*R*, 3*R*, 4*S*, 7*S*)-UK-2A. Isobutyric acid (3*S*,6*S*,7*R*,8*R*)-8-benzyl-3-(2-hydroxy-3-methoxybenzoylamino)-6-methyl-4,9-dioxo-[1,5]dioxonane-7-yl ester (28) (128 mg, 66 % from (*S*)-10) was obtained as a colorless crystalline solid (m.p. 78 ~ 83 °C);  $[\alpha]^{25}_{\text{D}} +94.9^\circ$  (c 0.1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ) 1.24 (d,  $J = 6.9$  Hz, 6H), 1.33 (d,  $J = 6.3$  Hz, 3H), 2.63 (septet,  $J = 6.9$  Hz, 1H), 2.70 (br d,  $J = 11.2$  Hz, 1H), 2.88–3.04 (m, 2H), 3.60 (m, 1H), 3.90 (s, 3H), 5.00 (dq,  $J = 9.6, 6.3$  Hz, 1H), 5.22 (t,  $J = 9.6$  Hz, 1H), 5.13–5.26 (m, 1H), 5.46 (m, 1H), 6.85 (t,  $J = 7.6$  Hz, 1H), 7.00 (d,  $J = 7.6$  Hz, 1H), 7.10–7.30 (m, 5H), 7.45 (br d,  $J = 6.6$  Hz, 1H), 10.76 (br s, 1H);  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ) 17.8 (q), 19.0 (q, 2C), 34.1 (d), 34.5 (t), 51.0 (d), 52.1 (d), 56.2 (q), 66.1 (t), 74.8 (d), 75.0 (d), 114.4 (s), 115.0 (d), 118.5 (d), 118.8 (d), 126.7 (d), 128.6 (d, 2C), 128.8 (d, 2C), 137.8 (s), 148.6 (s), 150.2 (s), 168.4 (s), 170.3 (s), 171.9 (s), 175.6 (s); IR (KBr) 3400 (br), 1750, 1645, 1585, 1540, 1460, 1365, 1250, 1180, 1140, 1070, 750  $\text{cm}^{-1}$ ; FABMS  $m/z$  514 (M+H)<sup>+</sup>; HRFABMS Calcd. for  $\text{C}_{27}\text{H}_{32}\text{NO}_9$ : 514.2077; Found: 514.2070.

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25. 4-Iodo-3-methoxymethoxypyridine was also prepared with 1,2-diiodo-1,1,2,2-tetrafluoroethane, but the next methoxy replacement was not as good as the case of 4-bromo-3-methoxymethoxypyridine because of the reductive deiodination by-reaction resulting in 3-methoxymethoxypyridine. It should also be noted that 4-bromo-3-methoxymethoxypyridine was much faster to react to NaOMe than 4-iodo-3-methoxymethoxypyridine, although the reason still remains unclear.
26. Structure of UK-2A is depicted with the absolute stereochemistry found in our synthetic UK-2A.
27. The physical data ( $[\alpha]_D$ , m.p., <sup>13</sup>C NMR, HRFABMS etc.) except the <sup>1</sup>H NMR, of  $\gamma$ -lactone **24** were completely identical with those of the reported  $\gamma$ -lactone **24**, but the <sup>1</sup>H NMR of the synthesized  $\gamma$ -lactone **24** was different from the reported one. The coupling pattern of each signal in our  $\gamma$ -lactone **24** was very similar to the reported  $\gamma$ -lactone **24**, however, the chemical shifts of some signals were not in accordance with those of the corresponding signals of the reported  $\gamma$ -lactone **24**. Since  $\gamma$ -lactone **25** synthesized in our hands was identical with the reported  $\gamma$ -lactone **25**, our  $\gamma$ -lactone **24** should be identical with the reported  $\gamma$ -lactone **24**.
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31. Though we have postulated it would be reasonable that the enolization process started from the most stable conformer of each substrate, there might be a possibility that the transformation occurred from the less stable conformer of the starting substrate to the less stable one of the terminal product.
32. The proton chemical shift at the C<sub>6'</sub> position has been reported as  $\delta$  6.67 ppm.<sup>10</sup> However, it was revised as  $\delta$  7.66 ppm in a private communication from Prof. M. Taniguchi. One of the proton chemical shifts at the C<sub>8</sub> position and the carbon chemical shift at the C<sub>8</sub> were not reported in the original paper<sup>10</sup> because of the small amount of natural UK-3A. In the private communication from Prof. M. Taniguchi and Dr. O. Sakanaka, they were reported as  $\delta$  3.08 ppm and  $\delta$  65.1 ppm, respectively.
33. This material exhibited a rotation of -16.2, which compares somewhat favorably with the reported rotation ( $[\alpha]^{25}_{\text{D}}$  -23.20° *c* 0.6, CHCl<sub>3</sub>) for the degradation product **25**. However, further confirmation of the enantiomeric purity was sought. When the synthesized  $\gamma$ -lactone **25** was subjected to the chiral HPLC examinations (Dacel Chiralcel OD-H, OB-H and OJ), we detected only one peak in every case. Furthermore, the  $[\alpha]^{25}_{\text{D}}$  of the synthetic precursor  $\gamma$ -lactone **24** was completely identical with that of the reported  $\gamma$ -lactone **24**. From these results along with the fact that our two starting materials for the C<sub>1</sub>-C<sub>4</sub> segment in the nine-membered dilactone were both chiral, we believe that the optical purity of our  $\gamma$ -lactone **25** is excellent.
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