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Synthesis and Structure-activity Relationship of Antifungal Coniothyriomycin Analogues

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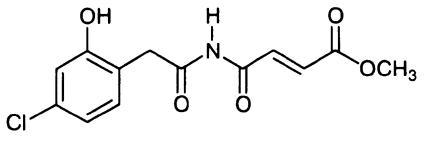
The structure of the antifungal metabolite coniothyriomycin was systematically modified by changing the acids of the open chain imide, modification of the hydrophobicity, variation in the degree of saturation, replacement of carbons by nitrogen or oxygen, and incorporation of the open chain molecule into cyclic arrangements. Structure-activity studies showed that antifungal activity was retained by replacement of phenylacetic acids by benzoic acids in the imide structure but diminished by hydrogenation of the fumaric ester part.

In connection with our investigation of fungal metabolites as biologically active agents, we isolated an open chain imide named coniothyriomycin (**1**) (Fig. 1) with remarkable antifungal activity from an unidentified *Coniothyrium* fungus[1]. In this communication, the synthesis of analogues with variation of the substituents on the aromatic ring and their biological activity were also presented. Unfortunately, these open chain mixed amides of phenylacetic and fumaric acid, in spite of excellent short-term antifungal activity, did not show curative effects. One reason for this was the inherent instability of the imide functionality in the presence of nucleophiles such as water. Therefore, in the hope to increase the chemical stability and retain or even increase antifungal activity, we extended our study to the preparation and biological testing of various analogues by (i) replacement of the substituted phenylacetic acids with substituted benzoic acids, (ii) change of hydrophobicity by variation of the alcohol component, (iii) variation in the degree of saturation of the fumaric acid moiety, (iv) replacement of carbon by nitrogen or oxygen in the middle part of the molecule, and (v) incorporation of the open chain part of the molecule into cyclic arrangements.

Fig. 1. Structure of coniothyriomycin (**1**).



Results and Discussion

In the previous syntheses of coniothyriomycin analogues[1] we used the method of SUHARA *et al.*[2] starting from nitriles *via* the corresponding ethyl imidates. However, the yields using this method were poor (10~36%) and the workup of the dark brown reaction mixture was tedious. Our first efforts were therefore directed towards a simplification of the procedure. To that end, equivalent amounts of benzamide (**2**) were heated with the fumaric monoethyl ester chloride (**3a**) in toluene (Scheme 1). However, starting with a 1 : 1 ratio, only modest yields of the desired mixed imide **5** resulted and the mixed anhydride **4** was identified as the major side product. Evidently, both theoretically possible reaction pathways a

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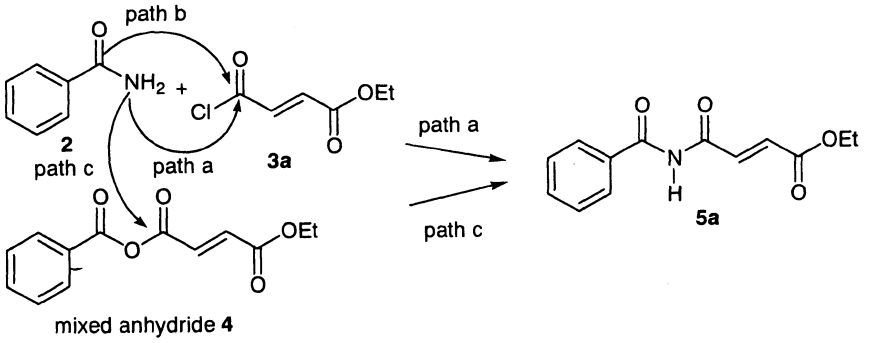
Scheme 1. Formation of mixed imide **5a** starting from amide **2** and acid chloride **3a** *via* the mixed anhydride **4**.

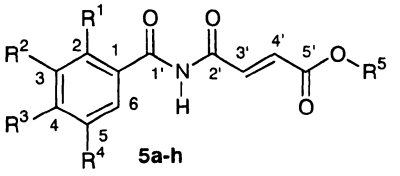


and b were realized: attack by the amide nitrogen on the acyl chloride (path a) to form **5a**, and attack by the nucleophilic oxygen (path b) to form 4. However, the acyl group transfer potential was still preserved in the mixed anhydride **4** and attack by excess benzamide (path c) on the mixed anhydride carbonyl might ultimately result in the formation of the mixed open chain imide **5a**. In fact, using two equivalents of amide **2**, pathway c was also followed consuming the mixed anhydride **4**, and yields of the mixed imide **5a** of 50% and more were achieved in a clean, easily worked-up reaction. Interestingly, the formation of the symmetric benzoic acid imide, resulting from attack of the nitrogen on the benzoic acid part of anhydride **4**, was not observed.

The method proved to be general and most of the mixed imides described in this paper were prepared using this simple procedure. However, in some cases it was advantageous to use the anion of amides such as **2**. Also, the yields were generally higher starting with benzamide (**2**) than with phenylacetic acid amide (**6a**).

The first series of compounds prepared were a number of mixed benzoic acid and fumaric ester open chain imides with variation in the substituents in the aromatic ring and the ester alcohol component. We wanted to see whether omission of the methylene group in **5a** by replacement of phenylacetic acid in the coniothriomycin analogues[1] with benzoic acid would preserve their antifungal activity. In addition, variation of the substituents from methoxy to fluoride or nitro groups as in **5b**~**5g** (Fig. 2) would show the influence of electron density on biological activity. Furthermore, the lipophilicity of the fungicide was increased in **5h** by linking the *n*-octanyl ester of fumaric

Fig. 2. Mixed benzoic acid-fumaric ester imides **5a**~**5h**.



acid chloride to benzamide. The data and substituents of the mixed benzoic acid-fumaric ester imides **5a**~**5h** are listed in Table 1.

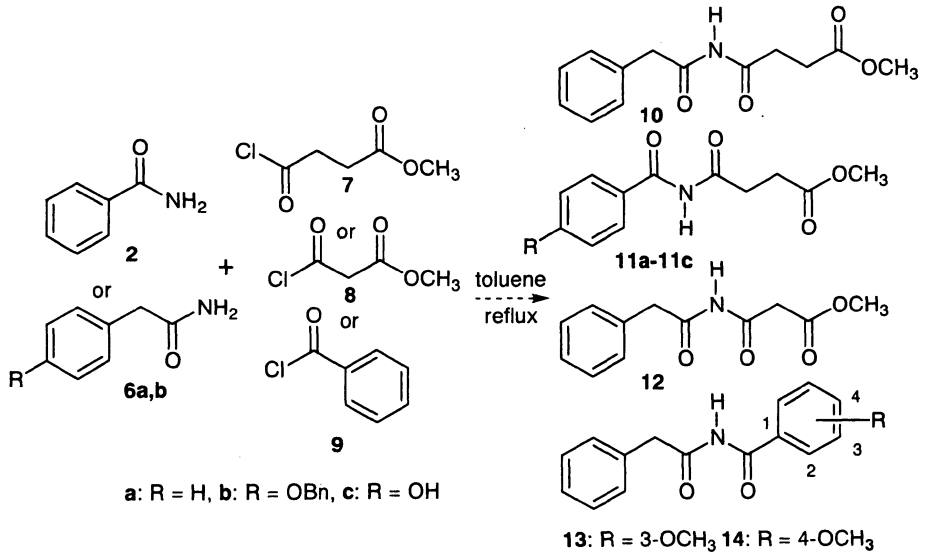
The next task was the variation in the degree of saturation in the fumaric ester part, starting from phenylacetic amide as well as benzoic acid amide. Omission of a methine group, *e.g.* the shift from fumaric ester to malonic ester, or replacement with benzoic acid (incorporating the double bond of fumaric acid into a ring system) was also in line with this type of variation and worth testing for fungicidal activity. The construction of the mixed imides **10**~**14** by coupling of amides **2** and **6a**, **b** with the acid chlorides **7**~**9** is shown in Scheme 2, employing about two equivalents of the respective amides as outlined in Scheme 1. The phenolic imide **11c**, as present in the natural product coniothyriomycin (**1**), was prepared by reaction of benzyl ether **6b** with **7**, followed by hydrogenolysis of benzyl ether **11b** to **11c** in order to evaluate the influence of a phenolic hydroxy group on activity.

Next, we investigated the replacement of carbon by

Table 1. Data and substituents of mixed benzoic acid-fumaric ester imides **5a**~**5h**

	R^1	R^2	R^3	R^4	R^5	mp (°C)	yield (%)
5a	H	H	H	H	C_2H_5	87–89 °C	51 %
5b	F	H	H	H	C_2H_5	54-55°C	42 %
5c	H	H	NO_2	H	C_2H_5	135-137°C	44 %
5d	OCH_3	H	H	H	C_2H_5	98-100°C	46 %
5e	H	H	OCH_3	H	C_2H_5	120-122°C	45%
5f	H	OCH_3	H	OCH_3	C_2H_5	162-164°C	43 %
5g	H	OCH_3	OCH_3	OCH_3	C_2H_5	114-116°C	65%
5h	H	H	H	H	n-C_8H_{15}	oil	33 %

Scheme 2. Construction of mixed imides **10**~**14** by coupling of amides **2** and **6a,b** with the acid chlorides **7**~**9**.



nitrogen or oxygen in the middle part of the molecule to study the effect of these exchanges on bioactivity. Three different types of compounds resulted from these exchanges: The *bis*-acylated hydrazine **16**, the acylated hydroxamide **18**, and the acylated phenylhydrazines **20a**~**20c**. The compounds were prepared by reaction of the hydrazide anion of **15**, the hydroxamic acid **17** or the phenylhydrazines **19a**,**b** with the acid chlorides **3** or **3c**, respectively, as outlined in Scheme 3.

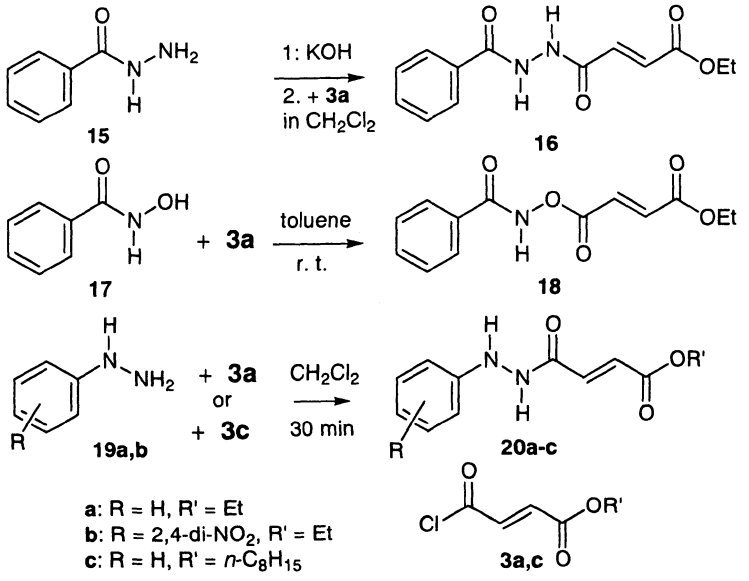
Finally, the imide function was incorporated in a cyclic arrangement, as demonstrated by the bold lines in structures **24**/**25** (Scheme 4). The 4-phenyl-pyrrolidine-2,3,5-trione (**23**) was prepared by sodium ethoxide mediated condensation of phenylacetic acid amide (**22**) with diethyl oxalate (**21**)[3]. The carbonyl group of **23** was then reacted with a number of Wittig ylides to afford the *E*-olefins **24**~**27** (compare[4]).

Biological Studies

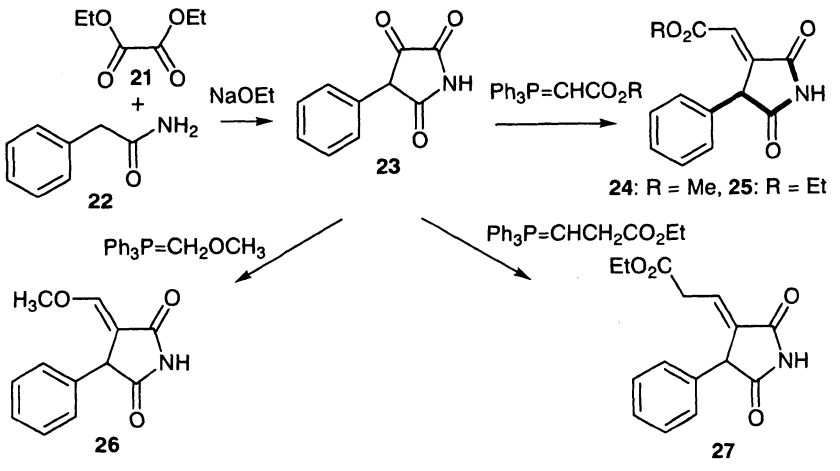
The tested synthetic coniothyriomycin analogues showed primarily control of plant diseases caused by representative fungi belonging to the class of Oomycetes, *e.g.* late blight on tomatoes caused by *Phytophthora infestans* or downy mildew on grape vine caused by *Plasmopara viticola*. The fungicidal activity was tested *in vitro* in 96-well microtitre plates and on intact plants in a greenhouse.

The change of the molecule fragment phenylacetic amide

Scheme 3. Synthesis of *bis*-hydrazide **16**, acyl hydroxamic acid **18** and fumaric hydrazides **20a~c**.



Scheme 4. Wittig reaction of 4-phenyl-pyrrolidine-2,3,5-trione (**23**) to yield the *E*-olefins **24~27**.



to benzoic amide in **5a** to **5h** retained the same good *in vitro* activity as the lead structure coniothyriomycin. **5a** to **5d** controlled *P. infestans* with ED_{90}-values of less than 0.5 ppm, **5e** even with less than 0.125 ppm. Some additional control of the causal organism of rice blast, *Pyricularia oryzae*, and of the causal organism of leaf blotch on wheat, *Septoria tritici* was observed for **5a** to **5e** with ED_{90}-values of less than 2 and 8 ppm respectively. The fungicidal *in vitro* activity could only partially be translated into *in vivo* activity in greenhouse tests on intact plants. Only **5f** showed some initial protective control of *P. infestans* on tomatoes with additional initial good protective activity against *P. viticola* on grapes. The compounds **5d**, **5g** and **5h** showed only some moderate control of *P. viticola*.

The hydrogenation of the double bond in the fumaric acid fragment, which resulted in **10** and **11a**, led to total loss of fungicidal activity both *in vitro* and *in vivo*. The other structural variations in the compounds **13** an **14**

resulted in reduced *in vitro* fungicidal activity against *P. infestans*, with ED_{90}-values of less than 31 and 8 ppm respectively. All other structural variations in the compounds **12**, **16**, **18**, **20a~c** and **24** led to a loss of any significant *in vitro* or *in vivo* fungicidal activity.

Experimental

Biological Tests

The *in vitro* tests were run in 96-well microtitre plates. The wells were filled with aqueous solutions of the compounds in the appropriate concentration prepared from a DMSO-stock solution. Thereafter the spore suspensions were added. The spores of *Botrytis cinerea*, *Pyricularia oryzae* and *Septori tritici* were suspended in aqueous malt extract at a final concentration of 2%, the spores of *Phytophthora infestans* in a 2% aqueous synthetic medium according to SCHEEPENS and FEHRMANN[5].

The plates were kept for 7 days at 18°C and constant humidity. Mycelial growth was assessed with a THERMOmax microplate reader from Molecular Devices at 405 nm and the values were related to the untreated check.

The *in vivo* tests were done on intact plants in a greenhouse. The plants were sprayed to run-off with an aqueous solution or suspension of the compound. The air-dried plants were inoculated the following day with aqueous spore suspensions of the appropriate fungus. The inoculated plants were then kept in growth chambers with high humidity and at temperatures favourable for the development of the plant disease. After 4 to 7 days, the disease development on the untreated checks had almost covered the whole leaf area. At this point in time the trials were assessed.

Chemical Synthesis

For general methods and instrumentation see[6].

Condensation of Amides with Acid Chlorides, General Procedure A and B

Procedure A: To a boiling solution of the amide (10 mmol) in dry toluene (20 ml) a solution of the acid chloride (5 mmol) in toluene (5 ml) was added dropwise. The mixture was refluxed overnight (TLC monitoring), the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (dichloromethane) and recrystallized from ether. For yields and mp of **5a~5h** see Table 1.

Procedure B: Alternatively, the sodium salt of the amide (prepared by reaction of the amide solution with sodium hydride) was employed, with fumaric acid monoethyl ester anhydride.

4-Benzoylamino-4-oxobut-2-enoic Acid Ethyl Ester (**5a**)

Benzamide (**2**) (1.23 g, 10 mmol) in dry toluene (20 ml) was reacted according to procedure A with fumaric acid monoethyl ester chloride (**3a**) (1.78 g, 7.5 mmol) in toluene (5 ml) to yield **5a** (1.18 g, 51%), mp: 87~89°C. IR (KBr) $\tilde{\nu}$=3291 cm^{-1} (N–H), 1724 (C=O), 1703 (C=O), 1672 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.36 (t, J=7 Hz, 3H, CH_3), 4.32 (q, J=7 Hz, 2H, CH_2), 6.90 (d, J=2 Hz, 2H, CH), 7.02 (d, J=2 Hz, 2H, CH), 7.61 (m, 3H, ArH), 7.96 (m, 2H, 5ArH), 9.43 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.6 (CH_3), 61.8 (CH_2), 128.5 (CH), 129.4 (CH), 133.3 (ArC), 134.1 (ArC), 134.3 (ArC), 135.2 (ArC), 165.4 (C=O), 166.3 (C=O), 167.3 (C=O). *Anal* Calcd for $C_{12}H_{11}NO_4$ (233.22): C 63.09, H 5.26; Found: C 61.66, H 5.29.

4-(2-Fluorobenzoylamino)-4-oxobut-2-enoïc Acid Ethyl Ester (**5b**)

2-Fluorobenzoic acid amide (2.000 g, 14.4 mmol) in dry toluene (15 ml) was reacted with fumaric acid chloride (**3a**) (1.300 g, 9.6 mmol) as described in procedure A to afford imide **5b** (890 mg, 42%), mp: 54~55°C. IR (KBr) $\tilde{\nu}$=3388 cm^{-1} (N–H), 1720 (C=O), 1705 (C=O), 1678 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.35 (t, J=7 Hz, 3H, CH_3), 4.30 (q, J=7 Hz, 2H, CH_2), 6.94 (d, J=14 Hz, 1H, CH), 7.38 (m, 2H, ArH), 7.61 (m, 1H, ArH), 7.88 (d, J=14 Hz, 1H, CH), 8.08 (m, 1H, ArH), 9.19 (d, J=13 Hz, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.5 (CH_3), 61.8 (CH_2), 116.9 (CH), 120.4 (ArC), 125.8 (CH), 132.7 (ArC), 134.7 (ArC), 135.4 (ArC), 135.8 (ArC), 158.5 (C=O), 162.5 (C=O), 163.5 (C=O), 165.6 (ArC). *Anal* Calcd for $C_{13}H_{12}FNO_4$ (265.24): C 58.87, H 4.56, N 5.28. Found: C 58.39, H 4.46, N 5.24.

4-(4-Nitrobenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (**5c**)

4-Nitrobenzoic acid amide (550 mg, 3.3 mmol) was reacted with fumaric acid chloride (**3a**) (200 mg, 1.5 mmol) as described in procedure A to afford imide **5c** (158 mg, 44%), mp: 135~137°C. IR (KBr) $\tilde{\nu}$=3244 cm^{-1} (N–H), 1720 (C=O), 1713 (C=O), 1678 (C=O). ^1H NMR (200 MHz, DMSO): δ=1.28 (t, J=7 Hz, 3H, CH_3), 4.24 (q, J=7 Hz, 2H, CH_2), 6.75 (d, J=16 Hz, 1H, CH), 7.54 (d, J=16 Hz, 1H, CH), 8.15 (d, J=9 Hz, 2H, ArH), 8.37 (d, J=9 Hz, 2H, ArH), 11.73 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO): δ=14.8 (CH_3), 59.6 (CH_2), 134.4 (CH), 137.8

(CH), 123.7 (ArC), 123.8 (ArC), 128.2 (ArC), 128.3 (ArC), 139.4 (ArC), 150.6 (ArC), 165.1 (C=O), 165.5 (C=O), 166.4 (C=O). *Anal* Calcd for $C_{13}H_{12}N_2O_6$ (292.24): C 53.43, H 4.14, N 9.59; Found: C: 55.29, H 4.53, N 8.56.

4-(2-Methoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (**5d**)

2-Methoxybenzoic acid amide (2.100 g, 13.9 mmol) was reacted with fumaric acid chloride (**3a**) (1.000 g, 7.4 mmol) as described in procedure A to afford imide **5d** (785 mg, 46%), mp: 98~100°C. IR (KBr) $\tilde{\nu}$=3303 cm^{-1} (N–H), 1720 (C=O), 1705 (C=O), 1684 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.38 (t, J=7 Hz, 3H, CH_3), 4.09 (s, 3H, OCH_3), 4.32 (q, J=7 Hz, 2H, CH_2), 6.94 (d, J=14 Hz, 1H, CH), 7.18 (m, 2H, ArH), 7.62 (m, 1H, ArH), 8.00 (d, J=14 Hz, 1H, CH), 8.24 (m, 1H, ArH), 10.44 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.5 (CH_3), 56.7 (OCH_3), 61.7 (CH_2), 112.2 (CH), 120.1 (ArC), 122.2 (CH), 133.3 (ArC), 135.6 (ArC), 136.3 (ArC), 136.9 (ArC), 158.2 (ArC), 164.2 (C=O), 165.6 (C=O), 166.4 (C=O). *Anal* Calcd for $C_{14}H_{15}NO_5$ (277.27): C 60.64, H 5.45, N 5.05; Found: C 60.38, H 5.47, N 5.19.

4-(4-Methoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (**5e**)

4-Methoxybenzoic acid amide (500 mg, 3.3 mmol) in dry toluene (8 ml) was reacted with fumaric acid chloride (**3**) (240 mg, 1.8 mmol) as described in A to afford **5e** (185 mg, 45%), mp: 120~122°C. IR (KBr) $\tilde{\nu}$=3271 cm^{-1} (N–H), 1726 (C=O), 1709 (C=O), 1668 (νC=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.38 (t, J=7 Hz, 3H, CH_3), 3.93 (s, 3H, OCH_3), 4.32 (q, J=7 Hz, 2H, CH_2), 6.98 (d, J=14 Hz, 1H, CH), 7.03 (d, J=8 Hz, 2H, ArH), 7.89 (d, J=8 Hz, 2H, ArH), 8.04 (d, J=14 Hz, 1H, CH), 8.70 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.5 (CH_3), 55.9 (OCH_3), 61.7 (CH_2), 114.6 (CH), 124.6 (ArC), 130.8 (CH), 133.8 (ArC), 135.4 (ArC), 136.7 (ArC), 137.5 (ArC), 164.4 (ArC), 165.5 (C=O), 165.7 (C=O), 167.5 (C=O). *Anal* Calcd for $C_{14}H_{15}NO_5$ (277.27): C 60.64, H 5.45, N 5.05; Found: C: 59.97, H: 5.62, N 5.22.

4-(3,5-Dimethoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (**5f**)

3,4-Dimethoxy-benzoic acid amide (500 mg, 2.8 mmol) in dry toluene (8 ml) was reacted with fumaric acid chloride (**3a**) (200 mg, 1.5 mmol) as described in procedure A to afford **5f** (362 mg, 43%), mp: 162~164°C. IR (KBr) $\tilde{\nu}$=3271 cm^{-1} (N–H), 1722 (C=O), 1705 (C=O), 1675 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.38 (t, J=7 Hz, 3H, CH_3), 3.88 (s, 6H, 2×OCH_3), 4.33 (q, J=7 Hz, 2H, CH_2), 6.72 (d, J=14 Hz, 1H, CH), 7.01 (s, 1H, ArH), 7.30 (d, J=14 Hz, 1H, CH), 8.02 (d, J=8 Hz, 2H, ArH), 8.78 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.5 (CH_3), 56.1 (OCH_3), 61.6 (OCH_2), 112.6 (CH), 124.8 (ArC), 130.4 (CH), 133.2 (ArC), 136.1 (ArC), 136.8 (ArC), 137.9 (ArC), 164.4 (ArC), 165.8 (C=O), 166.1 (C=O), 167.3 (C=O). *Anal* Calcd for $C_{15}H_{17}NO_6$ (307.30): C 58.63, H 5.58, N 4.56. Found: C 59.77, H 5.52.

4-(3,4,5-Trimethoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (**5g**)

The sodium salt of the amide was prepared by stirring a mixture 3,4,5-trimethoxy-benzoic acid amide (4 g, 18.9 mmol) and NaH (1 g, 20.8 mmol) in THF (100 ml) for 30 minutes, and reacted with fumaric acid monoethyl ester anhydride (5.1 g, 18.9 mmol) in 30 ml THF (procedure B). After column chromatography, **5g** was isolated (4.1 g, 65%). mp: 114~116°C. IR (KBr) $\tilde{\nu}$=3255 cm^{-1} (N–H), 1729 (C=O), 1707 (C=O), 1672 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.38 (t, J=7 Hz, 3H, CH_3), 3.97 (s, 9H, 3×OCH_3), 4.32 (q, J=7 Hz, 2H, CH_2), 6.94 (d, J=14 Hz, 1H, CH), 7.21 (s, 2H, ArH), 8.05 (d, J=14 Hz, 1H, CH), 9.42 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.5 (CH_3), 56.6 (OCH_3), 56.7 (OCH_3), 61.3 (OCH_3), 61.9 (OCH_2), 106.2 (CH), 124.7 (ArC), 127.1 (CH), 133.6 (ArC), 135.3 (ArC), 143.2 (ArC), 153.3 (ArC), 165.2 (ArC), 166.0 (C=O), 168.5 (C=O), 169.2 (C=O). *Anal* Calcd for $C_{16}H_{19}NO_7$ (337.32): C 56.97, H 5.68. Found: C 56.91, H 6.02.

4-Benzoylamino-4-oxobut-2-enoic Acid Octyl Ester (**5h**)

The sodium salt of benzamide was prepared by stirring a mixture of benzamide (500 mg, 4.1 mmol) and NaH (172 mg, 4.1 mmol) in THF (35 ml) for 30 minutes, and reacted with fumaric acid monooctyl ester anhydride (1.82 g, 4.1 mmol) in 15 ml THF (procedure B). After column chromatography, **13** was isolated as an oil (190 mg, 13%). IR (KBr) $\tilde{\nu}$=3292 cm^{-1} (N–H), 1724 (C=O), 1709 (C=O), 1687 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=0.91 (t, J=6 Hz, 3H, CH_3), 1.31 (m, 10H, CH_2), 4.25 (q, J=3 Hz, 2H, CH_2), 6.95 (d, J=14 Hz, 1H, CH), 7.55 (m, 2H, ArH), 7.68 (m, 1H, ArH), 7.98 (m, 2H, ArH), 8.06 (d, J=14 Hz, 1H, CH), 9.02 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.5 (CH_3), 23.1 (CH_2), 26.3 (CH_2), 28.9 (CH_2), 29.6 (CH_2), 32.2 (CH_2), 66.1 (OCH_2), 112.6 (CH), 128.4 (ArC), 129.4 (CH), 132.6 (ArC), 134.0 (ArC), 134.3 (ArC), 135.2 (ArC), 165.6 (ArC), 166.3 (C=O), 166.7 (C=O), 167.0 (C=O). *Anal* Calcd for $C_{19}H_{25}NO_4$ (331.41): C 68.86, H 7.60; Found: C 69.67, H 7.51.

4-Oxo-4-phenylacetylaminobutyric Acid Methyl Ester (**10**)

Phenylacetic acid amide (**6a**) (1.35 g, 10 mmol) in dry toluene (20 ml) was reacted with succinic acid monomethyl ester chloride (**7**)[7] (1.65 g, 12.26 mmol) as described in general procedure A to afford 692 mg (28%) of **10**. IR (KBr): $\tilde{\nu}$=3260 cm^{-1}, 1735 (C=O), 1705 (C=O), 1657 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=2.69 (t, J=2 Hz, 4H, CH_2), 3.69 (s, 2H, $PhCH_2$), 3.70 (s, 3H, OCH_3), 7.37 (m, 5H, ArH), 9.05 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=28.7 (CH_2), 28.9 (CH_2), 39.3 ($PhCH_2$), 50.4 (CH_3), 127.4 (ArC), 129.0 (ArC), 129.8 (ArC), 135.9 (ArC), 170.7 (C=O), 172.0 (C=O), 175.2 (C=O). *Anal* Calcd for $C_{13}H_{15}NO_4$ (249.26): C 62.58, H 6.01; Found: C: 61.33, H: 5.33.

4-Benzoylamino-4-oxobutyric Acid Methyl Ester (**11a**)

Benzamide (**2**) (1.23 g, 10 mmol) was reacted with succinic acid monomethyl ester chloride (**7**) (1.65 g, 12.26 mmol) in toluene (5 ml) according to general procedure A to afford **11a** (1.06 g, 43%). IR (KBr) $\tilde{\nu}$=3291 cm^{-1} (N–H), 1740 (C=O), 1709 (C=O), 1677 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=2.72 (t, J=4 Hz, 2H, CH_2), 3.34 (t, J=4 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 7.61 (m, 3H, ArH), 7.93 (m, 2H, ArH), 9.35 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=28.6 (CH_2), 33.3 (CH_2), 52.3 (OCH_3), 128.3 (ArC), 129.3 (ArC), 132.9 (ArC), 133.6 (ArC), 166.2 (C=O), 173.4 (C=O), 175.7 (C=O). *Anal* Calcd for $C_{12}H_{13}NO_4$ (235.24): C 61.22, H 5.53; Found: C 61.23, H 5.45.

4-(4-Hydroxybenzoylamino)-4-oxobutyric Acid Ethyl Ester (**11c**)

A solution of 4-benzyloxybenzamide (500 mg, 2.2 mmol) in dry THF (10 ml) was treated with NaH (88 mg, 2.2 mmol) and stirred for 1 hour at 20°C. Fumaric acid monoethyl ester anhydride (600 mg, 2.2 mmol) was added and the mixture was heated overnight (procedure B). The product was purified by column chromatography on silica gel (CH_2Cl_2) and crystallized from ether. A sample of the resulting benzyl ether (150 mg) was dissolved in dry THF (10 ml) and hydrogenated over Pd-C (10%) for 3 hours. The reaction mixture was filtered and the solvent removed at reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2) to yield **11c** (76 mg, 69%), mp: 146~148°C. IR (KBr) $\tilde{\nu}$=3091 cm^{-1} (N–H), 1715 (C=O), 1675 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.31 (t, 3H, CH_3), 2.76 (t, J=6 Hz, 2H, CH_2), 3.78 (t, J=6 Hz, 2H, CH_2), 4.22 (q, 2H, CH_2), 7.76 (d, J=7 Hz, 2H, ArH), 8.11 (d, J=7 Hz, 2H, ArH), 8.62 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.1 (CH_3), 29.2 (CH_2), 29.6 (CH_2), 61.7 (CH_2), 116.5 (ArC), 117.0 (ArC), 125.9 (ArC), 128.8 (ArC), 129.7 (ArC), 161.1 (ArC), 168.2 (C=O), 172.3 (C=O), 172.4 (C=O). *Anal* Calcd for $C_{13}H_{13}NO_5$ (263.25): C 59.31, H 4.98; Found: C 59.38, H 4.95.

3-Oxo-3-phenylacetylaminopropionic Acid Methyl Ester (**12**)

Phenylacetic acid amide (**6a**) (1.0 g, 7.4 mmol) was reacted with malonic acid monomethyl ester chloride (**8**)[8] (1.22 g, 8.1 mmol) in toluene (5 ml) to afford **12** (520 mg, 30%) after column chromatography (petroleum ether/ethyl acetate 3/1), mp 93~96°C. IR (KBr) $\tilde{\nu}$=3266 cm^{-1} (N–H), 1750 (C=O), 1735 (C=O), 1698.2 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=3.76 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.85 (s, 2H, $ArCH_2$), 7.31 (m, 3H, ArH), 7.40 (m, 2H, ArH), 8.62 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=44.2 (CH_2), 44.8 (CH_2), 53.1 (OCH_3), 128.3 (ArC), 129.6 (ArC), 129.9 (ArC), 135.9 (ArC), 170.7 (C=O), 171.8 (C=O). *Anal* Calcd for $C_{12}H_{13}NO_4$: (235.24): C 61.22, H 5.53; Found: C 60.85, H 5.28.

3-Methoxy-*N*-phenylacetylbenzamide (**13**)

Phenylacetic acid amide (**6a**) (1.35 g, 10 mmol) in dry toluene (20 ml) was reacted with 3-methoxybenzoic acid chloride (1.26 g, 9.4 mmol) as described in procedure A to afford imide **13** (926 mg, 35%), mp: 112~114°C. IR (KBr) $\tilde{\nu}$=3312 (N–H) cm^{-1}, 1709 (C=O), 1683 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=3.86 (s, 3H, OCH_3), 4.36 (s, 2H, $PhCH_2$), 7.17 (t, J=2 Hz, 1H, ArH), 7.37 (m, 8H, ArH), 9.05 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=44.3 (CH_2), 55.9 (OCH_3), 113.0 (ArC), 119.9 (ArC), 120.2 (ArC), 127.6 (ArC), 129.0 (ArC), 130.2 (ArC), 130.4 (ArC), 134.1 (ArC), 134.4 (ArC), 160.4 (ArC), 167.7 (C=O), 175.2 (C=O). *Anal* Calcd for $C_{16}H_{15}NO_3$ (269.30): C 71.29, H 5.57; Found: C 71.35, H: 5.54.

4-Methoxy-*N*-phenylacetylbenzamide (**14**)

Phenylacetic acid amide (**6a**) (1.35 g, 10 mmol) was reacted with 4-methoxybenzoic acid chloride (1.26 g, 9.4 mmol) as described in procedure A to afford imide **14** (807 mg, 30%), mp: 162~164°C. IR (KBr) $\tilde{\nu}$=3462 cm^{-1} (N–H), 1786 (C=O), 1601 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=3.88 (s, 3H, OCH_3), 4.37 (s, 2H, $PhCH_2$), 6.95 (t, J=5 Hz, 2H, ArH), 7.37 (m, 5H, ArH), 7.60 (d, J=4 Hz, 2H, ArH), 7.86 (d, J=4 Hz, 2H, ArH), 8.12 (m, 5H, ArH), 9.45 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=39.3 (CH_2), 56.0 (OCH_3), 114.2 (ArC), 125.8 (ArC), 127.4 (ArC), 128.3 (ArC), 129.0 (ArC), 129.8 (ArC), 135.9 (ArC), 165.4 (ArC), 167.7 (C=O), 170.7 (C=O). *Anal* Calcd for $C_{16}H_{15}NO_3$ (269.30): C 71.29, H 5.57; Found: C

71.34, H: 5.54.

4-(*N'*-Benzoylhydrazino)-4-oxobut-2-enoic Acid Ethyl Ester (**16**)

The potassium salt of benzoylhydrazine was prepared by reaction of benzoylhydrazine hydrochloride (300 mg, 2.2 mmol) in a hot methanolic solution (5 ml) of KOH (98 mg) and evaporation of the methanol at reduced pressure. The residue was dissolved in CH_2Cl_2 (10 ml) and fumaric acid chloride ethyl ester (360 mg, 2.7 mmol) was added. The solution was stirred at room temperature for 1 hour, the precipitate was filtered off and washed with cold CH_2Cl_2 to yield **16** (256 mg, 50%), mp: 236~238°C. IR (KBr) $\tilde{\nu}$=3498 cm^{-1} (N–H), 3390 (N–H), 1654 (C=O), 1650 (C=O). ^1H NMR (200 MHz, DMSO): δ=1.26 (t, 3H, CH_3), 4.20 (q, 2H, CH_2), 6.72 (d, J=15 Hz, 1H, CH), 7.09 (d, J=15 Hz, 1H, CH), 7.58 (m, 3H, ArH), 7.92 (m, 2H, ArH), 10.53 (s, 1H, NH), 11.15 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO): δ=14.9 (CH_3), 61.7 (CH_2), 128.3 (ArC), 129.4 (ArC), 130.8 (ArC), 131.5 (ArC), 132.7 (ArC), 133.4 (ArC), 135.4 (CH), 137.5 (CH), 161.4 (C=O), 165.6 (C=O), 166.7 (C=O). *Anal* Calcd for $C_{13}H_{14}N_2O_4$ (262.26) C: 59.54, H: 5.38; Found: C 59.48, H 5.34.

4-Benzoylaminoxy-4-oxobut-2-enoic Acid Ethyl Ester (**18**)

A mixture of benzoic acid (6.10 g, 50 mmol), hydroxylamine (65 mmol) and dicyclohexyl carbodiimide (10.30 g, 50 mmol) in methanol (30 ml) was stirred for 1 hour. The methanol was evaporated at reduced pressure and the residue extracted with 10% aqueous NaOH (10 ml). The basic phase was acidified with 10% HCl (10.3 ml) and extracted with CH_2Cl_2 (30 ml). The organic phase was dried (Na_2SO_4) and the solvent removed at reduced pressure. A sample of this crude hydroxamic acid **17** (250 mg), fumaric acid ethyl ester (**3a**) (300 mg, 1.8 mmol), and triethylamine (184 mg, 1.8 mmol) was dissolved in toluene (20 ml) and stirred for 1 hour at room temperature to yield **18** (290 mg, 61%) after column chromatography on silica gel, mp: 76~78°C. IR (KBr): $\tilde{\nu}$=3076 cm^{-1} (N–H), 1796 (C=O), 1720 (C=O), 1634 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.28 (t, 3H, CH_3), 4.24 (q, 2H, CH_2), 6.84 (d, J=13 Hz, 1H, CH), 6.98 (d, J=13 Hz, 1H, CH), 7.48 (m, 2H, ArH), 7.59 (m, 1H, ArH), 8.09 (m, 2H, ArH), 9.13 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.4 (CH_3), 61.9 (CH_2), 128.8 (ArC), 129.3 (ArC), 130.5 (ArC), 131.7 (ArC), 132.4 (ArC), 133.5 (ArC), 135.5 (CH), 137.5 (CH), 162.7 (C=O), 169.8 (C=O), 172.3 (C=O). *Anal* Calcd for $C_{13}H_{13}NO_5$ (263.25): C 59.31, H 4.98; Found: C 60.03, H 5.03.

3-(*N'*-Phenylhydrazinocarbonyl)-acrylic Acid Ethyl Ester (**20a**)

A mixture of phenylhydrazine (1.08 g, 10 mmol) and fumaric acid chloride ethyl ester (1.34 g, 10 mmol) in dichloromethane (25 ml) and 2~3 drops of triethyl amine was stirred for 30 minutes. The precipitate was filtered off and washed with CH_2Cl_2 to yield **20a** (976 mg, 48%), mp: 140~142°C. IR (KBr): $\tilde{\nu}$=3368 cm^{-1} (N–H), 3096 (N–H), 1644 (C=O), 1614 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.27 δ (t, J=7 Hz, 3H, CH_3), 4.22 (q, J=7 Hz, 2H, CH_2), 6.77 (d, J=14 z, 1H, CH), 6.96 (m, 2H, ArH), 7.27 (d, J=14 Hz, 1H, CH), 7.49 (m, J=8 Hz, 3H, ArH), 9.98 (s, 1H, NH), 10.15 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=13.8 (CH_3), 61.4 (OCH_2), 113.2 (ArC), 113.3 (ArC), 119.1 (ArC), 130.0 (ArC), 131.2 (ArC), 135.1 (CH), 137.8 (CH), 144.6 (ArC), 165.2 (C=O), 166.4 (C=O). *Anal* Calcd for $C_{12}H_{14}N_2O_3$ (234.25): C 61.53, H 6.02; Found: C 61.60, 6.08.

3-(*N'*-(2,4-Dinitrophenyl)-hydrazinocarbonyl]-acrylic Acid Ethyl Ester (**20b**)

A mixture of 2,4-dinitrophenylhydrazine (1.5 g, 7.6 mmol) and fumaric acid chloride ethyl ester (1.13 g, 8.4 mmol) in dichloromethane (30 ml) and 2~3 drops of triethyl amine was stirred for 30 minutes. The precipitate was filtered off, and washed with CH_2Cl_2 to yield **20b** (604 mg, 78%), mp: 161~163°C. IR (KBr) $\tilde{\nu}$=3278 cm^{-1} (N–H), 3024 (N–H), 1695 (C=O), 1670 (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ=1.28 δ (t, J=7 Hz, 3H, CH_3), 4.24 (q, J=7 Hz, 2H, CH_2), 6.75 (d, J=14 Hz, 1H, CH), 7.16 (d, J=14 Hz, 1H, CH), 7.56 (s, 1H, ArH), 8.37 (m, 2H, ArH). 10.08 (s, 1H, NH), 10.28 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=14.8 (CH_3), 61.8 (OCH_2), 110.0 (ArC), 113.5 (ArC), 134.4 (ArC), 135.2 (CH), 137.6 (CH), 143.5 (ArC), 148.9 (ArC), 150.1 (ArC), 165.0 (C=O), 166.1 (C=O). *Anal* Calcd for $C_{12}H_{12}N_4O_7$ (324.25): C 44.45, H 3.73; Found: C 44.36, 3.69.

3-(*N'*-Phenyl)-hydrazinocarbonyl-acrylic Acid Octyl Ester (**20c**)

A solution of phenylhydrazine (504 mg, 4.6 mmol) and fumaric acid monooctyl ester anhydride (1.15 g, 5.0 mmol) in CH_2Cl_2 (10 ml) and 2~3 drops of triethyl amine was stirred at room temperature for 1 hour. The solution was cooled to 0°C, the precipitate was filtered off and washed with cold CH_2Cl_2 to yield hydrazide **20c** (800 mg, 57%), mp: 130~132°C. IR (KBr): $\tilde{\nu}$=3383 cm^{-1} (N–H), 3283 (N–H), 1675 (C=O), 1594 (C=O). ^1H NMR (200 MHz,

$CDCl_3$): δ=0.91 (t, 3H, CH_3), 1.32 (m, 10H, 5×CH_2), 4.23 (t, J=4 Hz, 2H, CH_2), 6.73 (d, J=14 Hz, 1H, CH), 7.01 (d, 2H, ArH), 7.21 (d, J=14 Hz, 1H, CH), 7.51 (m, 3H, ArH), 9.88 (s, 1H, NH), 10.11 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ=13.9 (CH_3), 22.8 (CH_2), 26.1 (CH_2), 29.8 (CH_2), 30.2 (CH_2), 30.5 (CH_2), 32.1 (CH_2), 65.4 (OCH_2), 112.9 (ArC), 118.7 (ArC), 129.1 (ArC), 133.8 (CH), 134.0 (CH), 134.2 (ArC), 165.0 (C=O), 165.7 (C=O). *Anal* Calcd for $C_{12}H_{12}N_4O_7$ (324.25): C 68.65, H 8.49; Found: C 68.05, 8.43.

(2,5-Dioxo-4-phenylpyrrolidin-3-ylidene)-acetic Acid Methyl Ester (**24**)

4-Phenylpyrrolidine-2,3,5-trione (**23**) was prepared by sodium ethoxide-catalyzed condensation of phenylacetamide (**22**) and ethyl oxalate (**21**) as described in the literature.[3] A solution of **24** (94.5 mg, 0.5 mmol) and methoxycarbonyl-methylenetriphenyl phosphorane (167 mg, 0.5 mmol) in dichloromethane (30 ml) was refluxed for 3 hours. The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel to yield **24** (84 mg, 69%), mp: 92~93°C. ^{1}H-NMR (200 MHz, $CDCl_3$): δ=3.52 (s, 3H, OCH_3), 4.69 (s, 1H, PhCH), 6.70 (s, 1H, CH), 7.37 (m, 5H, ArH). ^{13}C-NMR (50 MHz, $CDCl_3$): δ=53.9 (CH_3), 125.8 (CH), 128.1 (ArC), 130.6 (ArC), 130.8 (ArC), 134.0 (ArC), 134.2 (ArC), 165.3 (C=O), 166.3 (C=O), 170.7 (C=O). *Anal* Calcd for $C_{13}H_{11}NO_4$ (245.23): C 63.67, H 4.52; Found: C 62.75, H 4.81.

(2,5-Dioxo-4-phenylpyrrolidin-3-ylidene)-acetic Acid Ethyl Ester (**25**)

A solution of 4-phenylpyrrolidine-2,3,5-trione (**23**) (510 mg, 2.7 mmol) and ethoxycarbonyl-methylene-triphenyl phosphorane (940 mg 2.7 mmol) in dichloromethane (30 ml) was refluxed for 3 hours. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel to afford ester **27** (386 mg, 55%), mp: 108~109°C. ^{1}H-NMR (200 MHz, $CDCl_3$): δ=1.04 (t, J=7.1 Hz, 3H, CH_3), 4.01 (q, J=7.1 Hz, 2H, OCH_2), 6.95 (s, 1H, CH), 7.66 (m, 5H, ArH). *Anal* Calcd for $C_{14}H_{13}NO_4$ (259.26): C 64.86, H 5.05; Found: C 63.92, H 5.36.

3-Methoxymethylene-4-phenylpyrrolidine-2,5-dione (**26**)

A solution of methoxymethylene-triphenylphosphonium chloride (911 mg, 2.7 mmol) in dry THF was treated at −78°C with potassium *tert*-butoxide (303 mg, 2.7 mmol). The solution was allowed to warm to 20°C, 4-phenylpyrrolidine-2,3,5-trione (**23**) (510 mg, 2.7 mmol) was added, and stirring was continued for 24 hours. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (dichloromethane/methanol=9/1) to afford the enol ether **26** (283 mg, 48%). ^{1}H-NMR (200 MHz, $CDCl_3$): δ=3.51 (s, 3H, OCH_3), 4.16 (s, 1H, PhCH), 5.25 (s, 1H, CH), 7.37 (m, 5H, ArH). *Anal* Calcd for $C_{12}H_{11}NO_3$ (217.22): C 66.35, H 5.10; Found: C 65.39, H 4.86.

3-(2,5-Dioxo-4-phenylpyrrolidin-3-ylidene)-propionic Acid Ethyl Ester (**27**)

A solution of ethoxycarbonyl-ethylene-triphenylphosphorane bromide in dry THF (20 ml) was treated at −78°C with *tert*-BuOK (303 mg, 2.7 mmol). After 15 minutes stirring, trione **23** (500 mg, 2.7 mmol) was added. The mixture was allowed to warm to room temperature and stirring was continued for 24 hours. The solvent was removed under reduced pressure. The residue was redissolved in CH_2Cl_2 and filtered and the product was purified by column chromatography on silica gel (dichloromethane/methanol 9/1) to yield the ester **27** (295 mg, 40%). ^{1}H-NMR (200 MHz, $CDCl_3$): δ=1.11 (t, J=7.1 Hz, 3H, CH_3), 3.76 (m, 2H, CH_2), 4.13 (q, J=7.1 Hz, 2H, OCH_2), 6.86 (s, 1H, CH), 7.66 (m, 5H, ArH). *Anal* Calcd for $C_{15}H_{15}NO_4$ (273.28): C 65.92, H 5.53; Found: C 64.12, H 5.05.

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