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Linear and Cyclic N-Acetyl-α-aryl-glycines: Synthesis and Chemiluminescence Studies

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Summary. A series of N-acetyl-α-arylglycines (**IIa**, **IIb**) was prepared by acid-induced electrophilic α-amidoalkylation reactions; compounds of type **IIb** were transformed into the corresponding 3-acetylaminobenzo[*b*]furan-2(3*H*)ones (**III**) by treatment with acetic anhydride. It was found that most of these new compounds (lactones as well as open chain derivatives) undergo base induced oxidation in the presence of oxygen with the emission of visible light. Preliminary structure-activity relationships for these novel chemiluminescence class are proposed.

Keywords. 3-Acetylaminobenzo[*b*]furan-2(3*H*)ones; N-Acetyl-α-arylglycines; α-Amidoalkylation; Chemiluminescence.

Lineare und cyclische N-Acetyl-α-aryl-glycine: Synthese und Chemilumineszenz-Untersuchungen

Zusammenfassung. Eine Reihe von N-Acetyl-α-arylglycinen (**IIa, IIb**) konnte durch sauer induzierte elektrophile α-Amidoalkylierung hergestellt werden; die Behandlung von Verbindungen des Typs **IIb** mit Acetanhydrid führte zu den entsprechenden 3-Acetylaminobenzo[*b*]furan-2(3*H*)onen (**III**). Es zeigt sich, daß die Mehrzahl dieser neuen Verbindungen (sowohl Lactone als auch offenkettige Derivate) bei basisch induzierter Oxidation mit (Luft-)Sauerstoff sichtbares Licht emittieren. Erste Struktur-Aktivitäts-Beziehungen für diese neue chemilumineszenzierende Klasse werden vorgeschlagen.

Introduction

Chemiluminescence is the emission of light (UV, visible or IR) as an additional effect of a chemical reaction; it occurs when a reaction product appears in an electronically excited state and reaches the ground state by emission of a photon. The majority of chemiluminescence reactions are weak, but there has been enormous progress in the development of more efficient systems for applications in analytical chemistry and medicinal diagnostics [1,2].

During our studies on electrophilic α-amidoalkylation of phenols, we observed that treatment of the resulting product 3-acetylamino-5-methylbenzo[*b*]furan-2(3*H*)one (**2c**) with an organic base in the presence of (air) oxygen caused emission of light. The chemiluminescence of related 3-alkoxycarbamoylbenzo[*b*]furan-2(3*H*)ones (**Ia**) have been mentioned in the literature, however, without any details about structure-activity relationships [3]. This encouraged us to synthesize a variety of substituted 3-acylaminobenzo[*b*]furan-2(3*H*)ones (**III**) *via* N-acyl-α-arylglycines

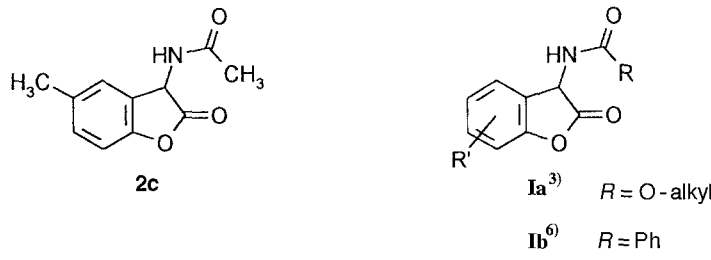


Fig. 1.

of type **IIa** and **IIb** as well as to investigate the structural features decisive for the chemiluminescence reaction.

Here, we report on the preparation of linear and cyclic N-acetyl-α-arylglycine derivatives of phenols and on preliminary chemiluminescence studies.

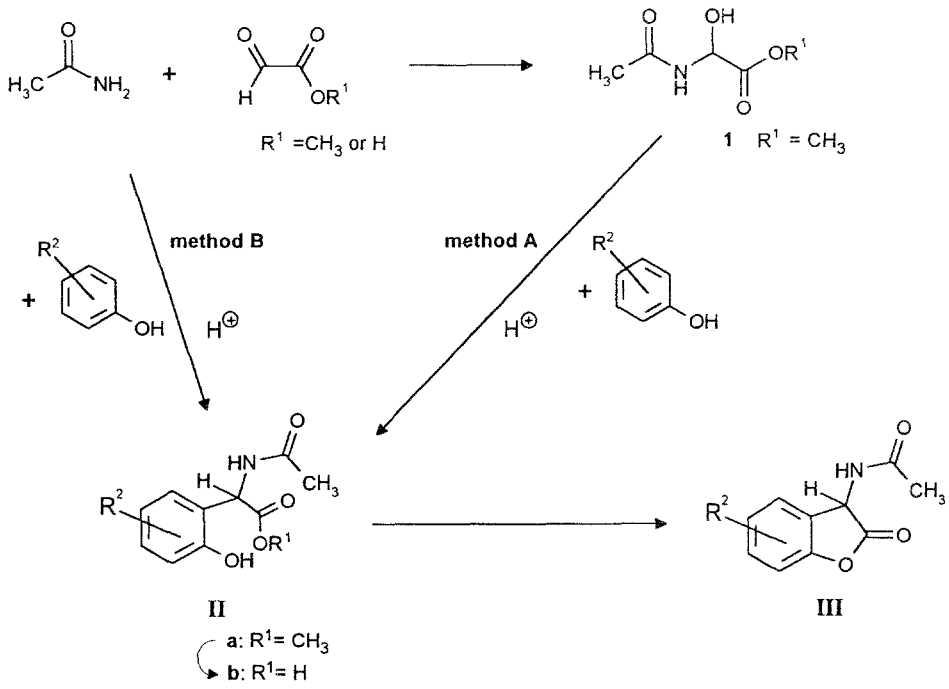
Results and Discussion

Starting from acetamide, glyoxylic acid monohydrate, or methyl glyoxylate, respectively, and substituted phenols, compounds of type **IIa** and **IIb** were prepared in a one-pot reaction (method B) or in a two step procedure *via* methyl N-acetyl-α-hydroxyglycinate (**1**, method A). Compound **1** was synthesized by refluxing acetamide and methyl glyoxylate in ethyl acetate [4]. The electrophilic α-amidoalkylation reactions were carried out in a mixture of formic acid containing traces of conc. sulfuric acid at room temperature. Only 4-nitrophenol required conc. sulfuric acid as reaction medium.

Employment of 2-benzylphenol yielded a mixture of *ortho* and *para* isomers (on the phenol part of the molecule) as indicated by proton NMR spectroscopy with the *para* isomer predominating by far. The pure *para* isomer could be separated by crystallization. α-Amidoalkylation reactions of diphenols (hydroquinone, resorcinol, and 2-methylresorcinol) under the same conditions (amidoalkylating agent:diphenol = 1:1) resulted in mixtures of the mono- and disubstituted products. This reaction, however, can be directed towards the monosubstituted product by using two equivalents of the diphenol. On the other hand, using two equivalents of the amidoalkylating agent results almost exclusively in the formation of the disubstituted products.

Heating carboxylic acids of type **IIb** (prepared by method B with glyoxylic acid or by alkaline hydrolysis of the corresponding esters **IIa**) in acetic anhydride yielded the substituted 3-acetylaminobenzo[*b*]furan-2(3*H*)ones **III**. In some cases, the lactones **III** were formed directly during the α-amidoalkylation reaction. This immediate transformation has also been observed for similar types of reactions [5].

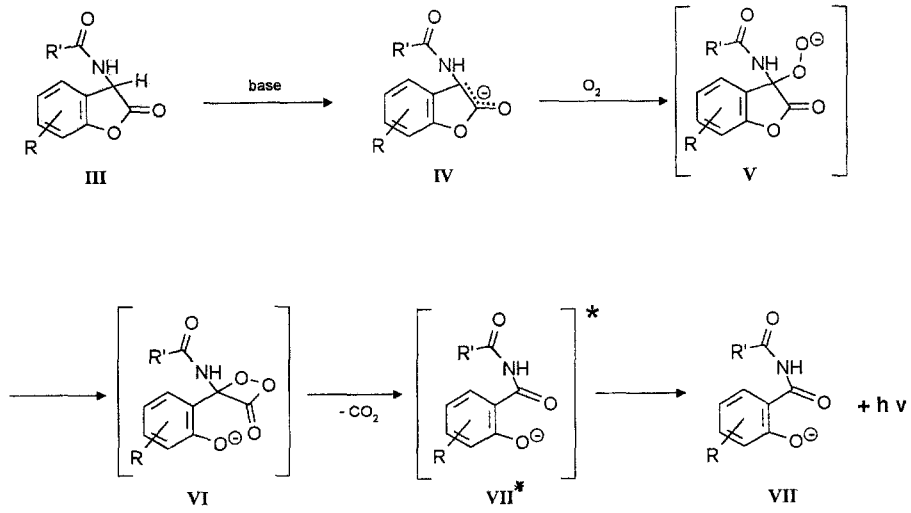
The spectroscopic data of all newly prepared compounds are in full agreement with the assigned structures. The ^{1}H NMR spectra of the lactones **III** (in *DMSO*-d_6) show a significant characteristic: D_2O-exchange occurs much faster with the α-CH proton than with the NH proton. This demonstrates the high mobility of the proton on the α-CH group, and thus we propose that the loss of this proton is the introductory step in the chemiluminescence reaction.



Scheme 1. Synthesis of *N*-acetyl-α-arylglycines **IIa** and **IIb** and of the corresponding 3-acetylaminobenzo[*b*]furan-2(3*H*)ones **III**

Chemiluminescence of the title compounds (*e.g.* **III**) can be observed upon treatment with base (preferably 1,8-diazabicyclo[5.4.0]undec-7-ene) in polar aprotic solvents (*e.g.* acetone) in the presence of air or oxygen. In contrast to the results of *Lofthouse et al.* [3] who postulate that an urethane substructure (**Ia**) is essential for the chemiluminescence reaction, we found that also compounds derived from primary carboxamids (including the benzamido derivative **Ib** [6]) undergo base-induced oxidation with (air)oxygen accompanied by emission of light. The postulation of *Lofthouse et al.* was based on the observation that **Ib** did not emitt visible light upon treatment with triethylamine in acetonitrile [3]. Based on their results, they suggested that a good leaving group in the molecule (RO^- in the case of urethanes) which allows the formation of a benzoxazinedione is essential for the emission of light [3].

These authors proposed a radical mechanism for the formation of a peroxy intermediate (V) during the chemiluminescence reaction. However, we found that neither a radical starter (*AIBN*) nor a radical inhibitor (1,4-hydroquinone) alter the intensity of the emitted light. Thus, an alternative mechanism for the chemiluminescence reaction of the title compounds is outlined in Scheme 2. The key step is the formation of the anion **IV** which reacts with oxygen to give the hydroperoxy anion **V**. It should be emphasized that ionic reactions with oxygen are well known [7]. Subsequently, the dioxetanone **VI** is formed by an intramolecular transformation of **V**. Release of carbon dioxide leads to **VII** in an electronically excited state which emits light upon relaxing to the ground state. In the chemiluminescence reaction using **2c**, the N-acetyl-2-hydroxy-5-methylbenzamide 1,8-diazabicyclo[5.4.0]undec-7-ene salt (**VII** with $R = 5\text{-}CH_3$ and $R' = CH_3$) was formed. The isolation and characterization of this salt is an additional proof for the mechanism proposed above.

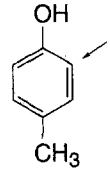
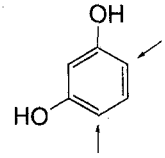


Scheme 2. Proposed mechanism for the chemiluminescence reaction

Interestingly, also the open chain compounds of type **IIa** and **IIb** were found to emit light under these conditions. To our knowledge, these linear N-acyl-α- aryl-glycines represent a novel class of chemiluminescent compounds.

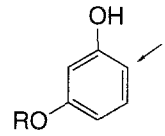
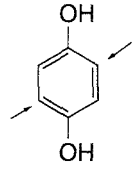
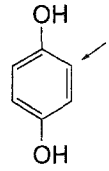
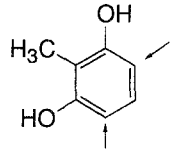
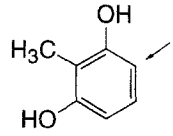
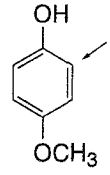
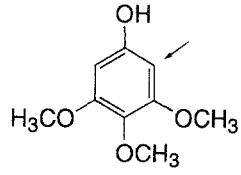
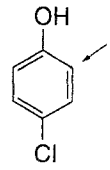
According to our observations, the colour of the emitted light varies depending on the substitution of the phenyl ring. Most compounds emit bluish light clearly visible in daylight. It should be noted that with the compounds obtained by α-amidoalkylation of diphenols (**3–8**), the initially occurring colour changes rapidly (see Table 1). This could be explained with the formation of two different fluorescent species (mono- and dianionic forms). Within the series studied, only compounds **12–14** do not show visible chemiluminescence.

Table 1. Chemiluminescence of compounds **2–14**

	Ar	Type	Colour and intensity of the emitted light[1]	Chemiluminescence[2] counts (quantity[3])
2a		**IIa**	blue-violet (*strong*)	3615 (10 nmol) 403890 (1 μmol)
2b		**IIb**	blue-violet (*weak*)	870 (10 nmol)
2c		**III**	blue-violet (*very strong*)	237228 (10 nmol) 37454 (1 nmol)
3		**IIa**	blue-violet turns to greenish (*weak*)	793 (10 nmol) 8862 (1 μmol)

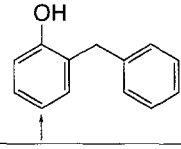
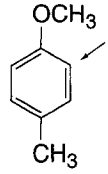
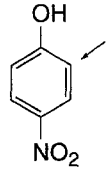
(*Continued*)

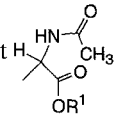
Table 1. (*Continued*)

	Ar	Type	Colour and intensity of the emitted light[1]	Chemiluminescence[2] counts (quantity[3])
4a (*R*=*H*)		**IIa**	violet turns to blue (*weak*)	793 (10 nmol) 8863 (1 μmol)
4b[8] (*R*=*H*)		**IIb**	violet turns to blue (*very weak*)	*
4c (*R*=$COCH_3$)		**III**	violet turns to blue (*weak*)	22988 (10 nmol) 588785 (1 μmol)
5		**IIa**	greenish turns to yellow ⇒orange ⇒ red (*weak*)	4673 (10 nmol)
6		**III**	turquoise turns to yellow (*weak*)	156501 (10 nmol)
7a		**IIa**	blue-violet turns to greenish (*weak*)	*
7b		**IIb**	blue-violet turns to greenish (*very weak*)	*
7c		**III**	blue-violet turns to greenish (*strong*)	*
8		**III**	violet turns to blue ⇒ yellow ⇒ orange (*weak*)	1300 (10 nmol) 26063 (1 μmol)
9		IIa	turquoise (*strong*)	20254 (10 nmol) 952433 (1 μmol)
10a		**IIa**	turquoise (*weak*)	382 (10 nmol) 2474 (1 μmol)
10b		**IIb**	turquoise (*very weak*)	*
10c		**III**	turquoise (*strong*)	2756 (10 nmol) 60434 (1 μmol)
11a		**IIa**	blue (*weak*)	683 (10 nmol)
11b		**III**	blue (*very strong*)	>999999 (10 nmol) 286344 (1 nmol)

(*Continued*)

Table 1. (*Continued*)

	Ar	Type	Colour and intensity of the emitted light[1]	Chemiluminescence[2] counts (quantity[3])
12		**IIa**	no visible chemiluminescence	*
13a		**IIa**	no visible chemiluminescence	903 (10 nmol) 1316 (1 μmol)
13b		**IIb**	no visible chemiluminescence	569 (10 nmol) 838 (1 μmol)
14		**IIa**	no visible chemiluminescence	577 (10 nmol)

↙ Position of the N-acetylglycine substituent 

[1] based on observation (reaction in acetone with 1,8-diazabicyclo[5.4.0]undec-7-ene and air oxygen); [2] based on measurement (reaction in acetonitrile and phosphate buffer (*pH* 7.8) with peroxidase and hydrogen peroxide [9]; [3] total quantity of the chemiluminecent compound in the reaction mixture; * compound not sufficient soluble in acetonitrile and/or the reaction medium

The following structure-activity relationships can be ascertained: the free carboxylic acids **IIb** show weak chemiluminescence, with intensity increasing in the order of the esters **IIa** and then the lactones **III** which show the strongest activity. In the series of the 3-acetylamino-2,3-dihydrobenzo[*b*]furan-2(3*H*)-ones **III**, additional substituents increasing the α-CH-acidity (*e.g.* R^2 = 5-Cl) intensify the chemiluminescence. In case of compounds **2–11** (type **IIa**, **IIb**), the phenolic hydroxyl function in *ortho* position to the amidoalkyl subunit causes increased acidity of the α-CH. Substituents on the benzene ring which increase the α-CH-acidity accompanied by a decrease in the OH-acidity also lead to enhanced light emission. This interpretation corresponds well with the *Hammett* values [10] of the substituents in these compounds. The lack of visible chemiluminescence of the nitro derivative **14** may be explained by mesomeric stabilization of the anion formed in the first step of the chemiluminescence reaction in this series.

Experimental

Melting points (uncorrected) were determined with a Linström apparatus; IR spetra (KBr pellets ν in cm^{-1}): Pye Unicam SP 3-200S; ^1H NMR spectra: Varian EM 360 (60 MHz, *TMS* as internal standard, δ in ppm); chemiluminescence measurement: Biolumat LB 9500C (Berthold). Elemental analyses:

Institute of Organic Chemistry and Biochemistry, University of Hamburg (Germany) and Institute of Physical Chemistry (Mag. *J. Theiner*), University of Vienna (Austria). Analytical TLC: Polygram® SIL G/UV_{254} (Macherey-Nagel) plastic-backed plates (0.25 mm layer thickness); light petroleum: fraction of *b.p.* 40 to 60°C.

Methyl N-acetyl-α-hydroxyglycinate (**1**) was prepared by refluxing a solution of 110 mmol of methyl glyoxylate (9.69 g) and 100 mmol of acetamide (5.91 g) in 25 ml of ethyl acetate for 3 h as described in the literature [4].

Reagents and solutions for chemiluminescence measurements

Peroxidase (*ca.* 250 U/mg): Boehringer Mannheim, No. 413 470.
Phosphate buffer 1 (*pH* 7.8): 13.6 g KH_2PO_4 were dissolved in *aqua bidest.*, 8 *N* NaOH was added until *pH* 7.8, and the solution was filled up with *aqua bidest.* to 100.0 ml.
Phosphate buffer II (*pH* 7.8): dilution of phosphate buffer I with *aqua bidest.* (1:100).
EDTA solution: 37.1 mg of *EDTA* disodium salt dihydrate were dissolved in 100 ml of *aqua bidest.*, the *pH* was brought up to 7.0 by slow addition of 1 *N* NaOH.
POD solution (2500 U/l): 1 mg of peroxidase lyophilisate were dissolved in 2.0 ml of phosphate buffer II; this solution was diluted 1:50 with phosphate buffer II.
H_2O_2 solution (*ca.* 1 m*M*): 10 µl conc. H_2O_2 solution diluted with 100.0 ml of *aqua bidest.*
Solutions of the chemiluminescent substance in acetonitrile:
a) (10 mmol/l): 0.05 mmol of **1** were dissolved in 5.0 ml of acetonitrile.
b) (100 µmol/l): Solution a) was diluted 1:100 with acetonitrile.
c) (10 µmol/l): Solution a) was diluted 1:1000 with acetonitrile.

Observation of chemiluminescence

A few mg of the appropriate compound were dissolved in acetone, and some drops of *DBU* were added. The colour and intensity of the emitted light was observed in a dark room.

Measurement of chemiluminescence [9]

200 µl of phosphate buffer I, 200 µl of *EDTA* solution, and 100 µl of *POD* solution were pipetted in a cuvette. After addition of 100 µl of the solution of chemiluminescent compound, 200 µl of H_2O_2 solution were injected and the maximum (mode of measurement: 'rate') was read.

Chemiluminescence products from **2c**

A mixture of **2c** (2 mmol) and *DBU* (2 mmol) in acetone (20 ml) was stirred at room temperature under an oxygen atomosphere for 3 days. The solution was treated with ether, and the resulting crystals were collected. The product (N-acetyl-2-hydroxy-5-methylbenzamide *DBU* salt) was recrystallized from tetrahydrofuran/light petroleum. Yield: 221 mg (32%) of colourless crystals; m.p.: 159–160°C; IR: 3050, 2950 (br, NH, NH_4^+), 1650 (CON); ^{1}H NMR (*DMSO*-d_6 + D_2O): $\delta = 1.64$–3.57 (m, 16H, 8 × CH_2), 2.07 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 6.19–7.48 (m, 3H, aromatic H); $C_{19}H_{27}N_3O_3$ (345.44); calcd.: C 66.06, H 7.88, N 12.16; found: C 65.82, H 7.96, N 12.19.

The filtrate was evaporated *in vacuo*; the residue was purified by column chromatography (Kieselgel 60, dichloromethane/ethyl acetate 5:2) to afford 39 mg (10%) of N-acetyl-2-hydroxy-5-methylbenzamide [11]. M.p.: 153–155°C (151–151.5°C[11]); IR: 3260 (NH), 3050 (br, OH), 1710 (weak), 1650 (CON); ^{1}H NMR (*DMSO*-d_6 + D_2O): $\delta = 2.25$ (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.81–7.75 (m, 3H, aromatic H).

Table 2. Analytical and physical data of compounds **2–14**

	Method	Yield	m.p.	Recryst. medium	C calcd. found	H calcd. found	N calcd. found	Formula
2a	A1	88%	203°C (decomp.)	ethyl acetate	60.75 60.74	6.37 6.38	5.90 6.00	$C_{12}H_{15}NO_4$ (237.26)
2b	C	87%	212–214°C	ethyl acetate	59.19 58.84	5.87 5.87	6.27 6.20	$C_{11}H_{13}NO_4$ (223.23)
2c	D	88%	202–203°C	tetrahydrofuran/ light petroleum	64.38 64.36	5.40 5.48	6.83 6.84	$C_{11}H_{11}NO_3$ (205.21)
3	A1 with 2 eq. of **1**	98%	256–258°C (decomp.)	H_2O	52.17 52.03	5.47 5.52	7.61 7.49	$C_{16}H_{20}N_2O_8$ (368.35)
4a	A1 with 2 eq. of resorcinol	49%	203–205°C	tetrahydrofuran	55.23 55.04	5.48 5.61	5.85 5.76	$C_{11}H_{13}NO_5$ (239.23)
001n **4b** [7]	C	76%	213–214°C Ref. [7]: 200°C	H_2O				
4c	D	49%	166–168°C	ethyl acetate	57.83 57.70	4.45 4.47	5.62 5.57	$C_{12}H_{11}NO_5$ (249.22)
5	A1 with 2 eq. of **1**	19%	298–300°C (decomp.)	DMSO/H_2O; dioxane/H_2O	52.17 52.12	5.47 5.39	7.61 7.58	$C_{16}H_{20}N_2O_8$ (368.35)
6	A1 with 2 eq. of hydraquinone	12%	244–245°C	H_2O	57.97 57.77	4.38 4.32	6.76 6.86	$C_{10}H_9NO_4$ (207.19)
7a	A1 with 2 eq. of **1**	86%	244–246°C (decomp.)	ethanol/H_2O	53.40 53.10	5.80 5.75	7.33 7.58	$C_{17}H_{22}N_2O_8$ (382.37)

7b	C	32%	208–211°C	H_2O	50.85 50.77	5.12 5.12	7.91 7.32	$C_{15}H_{18}N_2O_8$ (354.32)
7c	D	67%	>300°C	tetrahydrofuran	56.60 56.57	4.43 4.55	8.80 8.51	$C_{15}H_{14}N_2O_6$ (318.29)
8	A1 with 2 eq. of 2-methylresorcinol	27%	239–240°C	H_2O	59.73 59.88	5.01 5.08	6.33 6.25	$C_{11}H_{11}NO_4$ (221.21)
9	A1	91%	143–145°C	ethyl acetate; tetrahydrofuran/ light petroleum	56.91 57.20	5.97 6.28	5.53 5.80	$C_{12}H_{15}NO_5$ (253.26)
10a	A1	45%	128–130°C	ethyl acetate	53.67 53.45	6.11 6.02	4.47 4.42	$C_{14}H_{19}NO_7$ (313.31)
10b	C	94%	200–201°C	tetrahydrofuran/ light petroleum	52.17 52.30	5.73 5.89	4.68 4.60	$C_{13}H_{17}NO_7$ (299.28)
10c	D	71%	194–195°C	tetrahydrofuran/ light petroleum	55.51 55.52	5.38 5.38	4.98 4.89	$C_{13}H_{15}NO_6$ (281.27)
11a	A1	78%	204–205°C	ethyl acetate/tetra- hydrofuran 1:1	51.27 51.50	4.69 4.50	5.44 5.20	$C_{11}H_{12}ClNO_4$ (257.68)
11b	B	75%	204–206°C	tetrahydrofuran/ light petroleum	53.23 53.41	3.57 3.46	6.21 6.18	$C_{10}H_8ClNO_3$ (225.63)
12	A1	83%	159–160°C	tetrahydrofuran/ light petroleum	69.00 69.16	6.11 6.15	4.47 4.52	$C_{18}H_{19}NO_4$ (313.36)
13a	A1	60%	115–117°C	tetrahydrofuran/ light petroleum	62.14 62.26	6.82 6.82	5.57 5.86	$C_{13}H_{17}NO_4$ (251.28)
13b	B	91%	217–218°C	tetrahydrofuran/ light petroleum	60.29 60.42	6.41 6.45	5.86 6.15	$C_{12}H_{15}NO_4 \times 0.1$ H_2O (239.06)
14	A2	45%	209–210°C	methanol	49.26 49.37	4.51 4.50	10.44 10.22	$C_{11}H_{12}N_2O_6$ (268.23)

Table 3. IR and ^{1}H NMR spectra of compounds **2–14**

	IR (cm^{-1})	^{1}H NMR (ppm, *DMSO*-d_6)
2a	3380 (NH), 3160 (br, OH), 1750 ($COOCH_3$), 1655 (CON)	1.90 (s, 3H, $COCH_3$), 2.20 (s, 3H, CH_3), 3.61 (s, 3H, $COOCH_3$), 5.68 (d, s after D_2O-exchange, 1H, CH), 6.68 (s, 3H, aromatic H), 8.38 (d, 1H, D_2O exchangeable, NH), 9.57 (s, 1H, D_2O exchangeable, OH)
2b	3500 (OH), 3390 (NH), 1950–2450 (COOH), 1725 (COOH), 1610 (CON)	1.88 (s, 3H, $COCH_3$), 2.20 (s, 3H, CH_3), 5.60 (d, s after D_2O-exchange, 1H, CH), 6.66–7.05 (m, 3H, aromatic H), 8.26 (d, 1H, D_2O exchangeable, NH), 9–11.5 (br, 2H, D_2O exchangeable, OH, COOH)
2c	3260 (NH), 1820 (lactone-CO), 1655 (CON)	1.90 (s, 3H, $COCH_3$), 2.30 (s, 3H, CH_3), 5.34 (d, 1H, D_2O exchangeable, CH), 7.11 (s, 3H, aromatic H), 9.12 (d, 1H, D_2O exchangeable, NH)
3	3330 (NH), 3100 (br, OH), 1735 ($COOCH_3$), 1620 (CON)	1.87 (s, 6H, $COCH_3$), 3.41 (s, 6H, $COOCH_3$), 5.51 (d, s after D_2O-exchange, 2H, CH), 6.41 (s, 1H, aromatic H), 6.93 (s, 1H, aromatic H), 8.18 (d, 2H, D_2O exchangeable, NH), 9.78 (s, 2H, D_2O exchangeable, OH)
4a	3410 (NH), 3240 (br, OH), 1725 ($COOCH_3$), 1660 (CON)	1.87 (s, 3H, CH_3), 3.56 (s, 3H, CH_3), 5.51 (d, s after D_2O-exchange, 1H, CH), 6.08–6.96 (m, 3H, aromatic H), 8.18 (d, 1H, D_2O exchangeable, NH), 9.28 (s, 1H, D_2O exchangeable, OH), 9.58 (s, 1H, D_2O exchangeable, OH)
4c	3320 (NH), 1810 (lactone-CO), 1760 (COOR), 1700 (CON)	1.87 (s, 3H, $COCH_3$), 2.27 (s, 3H, CH_3), 5.33 (d, 1H, D_2O exchangeable, CH), 6.77–7.38 (m, 3H, aromatic H), 9.10 (d, 1H, D_2O exchangeable, NH)
5	3360 (NH), 3200 (br, OH), 1740 ($COOCH_3$), 1640 (CON)	1.85 (s, 6H, $COCH_3$), 3.59 (s, 6H, $COOCH_3$), 5.55 (d, s after D_2O-exchange, 2H, CH), 6.58–6.68 (m, 2H, aromatic H), 8.39 (d, 2H, D_2O exchangeable, NH), 9.23 (s, 2H, D_2O exchangeable, OH)
6	3310 (NH), 3180 (br, OH), 1810/1790 (lactone-CO), 1615 (CON)	1.89 (s, 3H, $COCH_3$), 5.29 (d, 1H, D_2O exchangeable, CH), 6.54–7.05 (s, 3H, aromatic H), 8.98 (d, 1H, D_2O exchangeable (fast), NH), 9.32 (s, 1H, D_2O exchangeable (slow), OH)
7a	3350 (NH), 3100 (br, OH), 1745 ($COOCH_3$), 1645 (CON)	1.87 (s, 6H, $COCH_3$), 2.05 (s, 3H, CH_3), 3.59 (s, 6H, $COOCH_3$), 5.58 (d, s after D_2O-exchange, 2H, CH), 6.76 (s, 1H, aromatic H), 8.36 (d, 2H, D_2O exchangeable, NH), 8.91 (s, 2H, D_2O exchangeable, OH)
7b	3400, 3300 (br, OH, NH), 1720 (COOH), 1615 (CON)	1.87 (s, 6H, $COCH_3$), 2.04 (s, 3H, CH_3), 5.48 (d, s after D_2O-exchange, 2H, CH), 6.48 (s, 1H, aromatic H), 8.22 (d, 2H, D_2O exchangeable, NH), 8.5–13 (br, 4H, D_2O exchangeable, OH, COOH)

(*Continued*)

Table 3. (*Continued*)

	IR (cm^{-1})	^1H NMR (ppm, *DMSO*-d_6)
7c	3340 (NH), 1830 (lactone-CO), 1650 (CON)	1.87 (s, 6H, $COCH_3$), 2.19 (s, 3H, CH_3), 5.33 (d, 2H, D_2O exchangeable, CH), 6.91 (s, 1H, aromatic H), 9.18 (d, 2H, D_2O exchangeable, 2 × NH)
8	3360 (NH), 3240 (br, OH), 1785 (lactone-CO), 1635 (CON)	1.85 (s, 3H, $COCH_3$), 2.04 (s, 3H, CH_3), 5.26 (d, 1H, D_2O exchangeable (fast), CH), 6.48–6.94 (m, 2H, aromatic H), 8.90 (d, 1H, D_2O exchangeable, NH), 9.68 (s, 1H, D_2O exchangeable, OH)
9	3410 (NH), 2960 (br, OH), 1755 ($COOCH_3$), 1640 (CON)	1.90 (s, 3H, $COCH_3$), 3.62 (s, 3H, CH_3), 3.69 (s, 3H, CH_3), 5.68 (d, s after D_2O-exchange, 1H, CH), 6.78 (s, 3H, aromatic H), 8.41 (d, 1H, D_2O exchangeable, NH), 9.32 (s, 1H, D_2O exchangeable, OH)
10a	3360 (NH), 2960 (br, OH), 1735 ($COOCH_3$), 1640 (CON)	1.87 (s, 3H, $COCH_3$), 3.59–3.74 ("m", 12H, CH_3), 5.81 (d, s after D_2O-exchange, 1H, CH), 6.26 (s, 1H, aromatic H), 7.90 (d, 1H, D_2O exchangeable, NH), 9.67 (s, 1H, D_2O exchangeable, OH)
10b	3340 (br, NH, OH), 1735 (COOH), 1615 (CON)	1.87 (s, 3H, $COCH_3$), 3.66–3.76 ("m", 9H, CH_3), 5.74 (d, s after D_2O-exchange, 1H, CH), 6.09 (s, 1H, aromatic H), 7.76 (d, 1H, D_2O exchangeable, NH), 9–12 (br, 2H, D_2O exchangeable, OH, COOH)
10c	3270 (NH), 1825 (lactone-CO), 1660, 1635 (CON)	1.84 (s, 3H, $COCH_3$), 3.67 (s, 3H, CH_3), 3.81 (s, 6H, CH_3), 5.39 (d, 1H, D_2O exchangeable, CH), 6.71 (s, 1H, aromatic H), 9.08 (d, 1H, D_2O exchangeable, NH)
11a	3370 (NH), 3140 (br, OH), 1745 ($COOCH_3$), 1645 (CON)	1.89 (s, 3H, $COCH_3$), 3.61 (s, 3H, $COOCH_3$), 5.71 (d, s after D_2O-exchange, 1H, CH), 6.76–7.28 (m, 3H, aromatic H), 8.53 (d, 1H, D_2O exchangeable, NH), 10.18 (s, 1H, D_2O exchangeable, OH)
11b	3310 (NH), 1830 (lactone-CO), 1650 (CON)	1.90 (s, 3H, $COCH_3$), 5.39 (d, 1H, D_2O exchangeable, CH), 7.16–7.58 (m, 3H, aromatic H), 9.18 (d, 1H, D_2O exchangeable, NH)
12	3380 (NH), 3220 (br, OH), 1730 ($COOCH_3$), 1620 (CON)	1.87 (s, 3H, $COCH_3$), 3.57 (s, 3H, $COOCH_3$), 3.86 (s, 2H, CH_2), 5.20 (d, s after D_2O-exchange, 1H, CH), 6.74–7.28 (m, 8H, aromatic H), 8.54 (d, 1H, D_2O exchangeable, NH), 7.5–9.8 (1H, D_2O exchangeable, OH)
13a	3320 (NH), 1755 ($COOCH_3$), 1650 (CON)	1.90 (s, 3H, $COCH_3$), 2.24 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 5.73 (d, s after D_2O-exchange, 1H, CH), 6.84–7.28 (m, 3H, aromatic H), 8.45 (d, 1H, D_2O exchangeable, NH)

Table 3. (*Continued*)

	IR (cm^{-1})	^1H NMR (ppm, *DMSO*-d_6)
13b	3340 (NH), 2940 (br, COOH), 1740 (COOH), 1620 (CON)	1.87 (s, 3H, $COCH_3$), 2.24 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 5.61 (d, s after D_2O-exchange, 1H, CH), 6.78–7.20 (m, 3H, aromatic H), 8.26 (d, 1H, D_2O exchangeable, NH) 10–13 (br 1H, D_2O exchangeable, COOH)
14	3380 (NH), 1730 ($COOCH_3$), 1610 (CON)	1.92 (s, 3H, $COCH_3$), 3.65 (s, 3H, $COOCH_3$), 5.84 (d, s after D_2O-exchange, 1H, CH), 6.98–8.16 (m, 3H, aromatic H), 8.72 (d, 1H, D_2O exchangeable, NH), 11.50 (s, 1H, D_2O exchangeable, OH)

Procedures for electrophilic α-amidoalkylation

Method A1

To a solution of 10 mmol of methyl N-acetyl-α-hydroxyglycinate (**1**, 1.47 g) in 10 ml of formic acid (98–100%), the appropriate phenol (10 mmol) was added. After addition of 5 drops of *conc.* sulfuric acid, the solution was stirred at room temperature for 3 days. The residue obtained after removal of formic acid was dissolved in 50 ml of ethyl acetate, and the organic layer was washed with water (3 × 20 ml), dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residues were recrystallized from appropriate solvents.

Method A2

To a solution of 10 mmol of methyl N-acetyl-α-hydroxyglycinate (**1**, 1.47 g) in 10 ml of conc. H_2SO_4, 4-nitrophenol (10 mmol) was added and the solution was stirred at room temperature for 5 days. Then, the reaction mixture was poured into 100 ml of ice water and was extracted with ethyl acetate (5 × 40 ml). The organic layer was washed twice with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was crystallized with diethyl ether and recrystallized from methanol.

Method B (one-pot reaction)

10 mmol of glyoxylic acid monohydrate (0.921 g), 10 mmol of acetamide (0.591 g), and 10 mmol of the appropriate phenolic compound were dissolved in a mixture of 10 ml of formic acid containing 5 drops of conc. sulfuric acid. After stirring at room temperature for 3 days, the reaction mixtures were treated as described under method A.

Hydrolysis (C)

To a suspension of 10 mmol of the methyl N-acetyl-α-arylglycinate (**2a**, **4a**, **7a**, or **10a**) in 15 ml of water, 11.5 mmol of sodium carbonate (1.219 g) were added and the mixture was refluxed for 2–5 min. After cooling the reaction mixture to 4°C, 6 *N* HCl was added until *pH* 1.

The compounds precipitating were filtered and washed with water, light petroleum, and diethyl ether. In cases where no precipitate was formed, the acidic solution was extracted with ethyl acetate. The organic layer was washed twice with water, dried over Na_2SO_4, and evaporated *in vacuo*. The residues were recrystallized from appropriate solvents.

Cyclization (general procedure, D)

15 mmol of the appropriate N-acetyl-α-(2-hydroxyaryl)-glycine (**2b**, **4b**, **7b**, or **10b**) were suspended in 8 ml of acetic anhydride and refluxed for some minutes. The solution was cooled and treated with 20 ml

of diethyl ether. The crystals precipitating were filtered off and washed with water, light petroleum, and diethyl ether. In cases where no precipitate is formed, the solution was evaporated *in vacuo*, the oily residue was dissolved in ethyl acetate, and the organic layer was washed twice with water. The ethyl acetate phase was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. Treatment of the residues with diethyl ether resulted in the formation of a precipitate. Analytically pure products could be obtained by recrystallization from appropriate solvents.

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