N-SALICYLIDENE DERIVATIVES OF PIRARUBICIN†

KEIICHI AJITO, DAISHIRO IKEDA, KEIKO KOMURO, CHISATO NOSAKA, NOBUKO WAKO, SHINICHI KONDO and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

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The preparation and biological evaluation of *N*-salicylidene derivatives of pirarubicin are described. Pirarubicin was treated with various kinds of aryl aldehydes. Most of compounds synthesized here were more active than pirarubicin *in vitro*. Some of them showed significant prolongation of the survival period in experimental mice by oral administration. Interestingly, a derivative containing forphenicine exhibited the broadest dose-response range by intraperitoneal administration.

An anthracycline antibiotic, doxorubicin, is widely used in the clinical treatment of leukemia and other tumors. This agent, however, has serious side effects, such as myelosuppression and irreversible cardiotoxicity. Pirarubicin, namely 4′-*O*-((*R*)-tetrahydropyranyl)adriamycin, prepared by UMEZAWA *et al.*[1] as a less cardiotoxic analogue of doxorubicin has recently been launched into clinical chemotherapy.

In the search for useful and orally absorbable analogues, we found that *N*-salicylidene derivatives of pirarubicin were therapeutically superior to doxorubicin in murine models. Some of them showed the possibility of being orally absorbable agents in *in vivo* tests.

We wish to demonstrate here the synthesis of sixteen *N*-salicylidene derivatives (**3**~**5** and **7**~**19**) of pirarubicin (**1**) and their biological evaluation *in vivo* and *in vitro*.

Chemistry

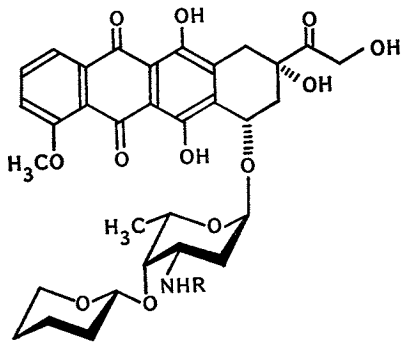
Pirarubicin (**1**) was treated with various kinds of aryl aldehydes in chloroform or in a mixture of chloroform and methanol. At first, **1** was converted into its *N*-salicylidene derivative (**3**) by treatment with salicylaldehyde in a good yield after chromatographic separation on silica gel with chloroform-methanol containing triethylamine. The SCHIFF base-methine proton of **3** in ^{1}H NMR spectrum resonated clearly at δ 8.35. A similar treatment of **1** with benzaldehyde did not afford a benzylidene derivative of **1** because of the lability of the product. *N*-Salicylideneadriamycin (**2**) was prepared by ARCAMONE *et al.*[2] as an intermediate of the synthesis of doxorubicin from daunorubicin, but they did not describe any biological data of **2**. Compound **3** was acylated with valeryl and decanoyl chlorides in pyridine and re-salicylidenated to give 14-*O*-valeryl and 14-*O*-decanoyl derivatives (**4** and **5**). To compare the biological activities of SCHIFF base derivative (**3**) with *N*-alkyl derivative, compound **6** was prepared by BORCH reduction of **1** with salicylaldehyde and $NaB(CN)H_3$ in acidic medium. Since the reductive alkylation of the amino group of **1** competed with the reduction of the 13-carbonyl group, the yield of **6** was poor.

N-Salicylidene derivatives of **1** having electron-donating group(s) on the aryl ring were synthesized. Reaction of **1** with substituted-aldehydes (3-, 4- and 5-hydroxy, 3,4-dihydroxy, 4- and 5-methoxy-

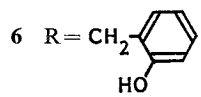
† A part of this work was presented at the 243rd JARA Research Meeting in Tokyo on Dec. 13, 1988.

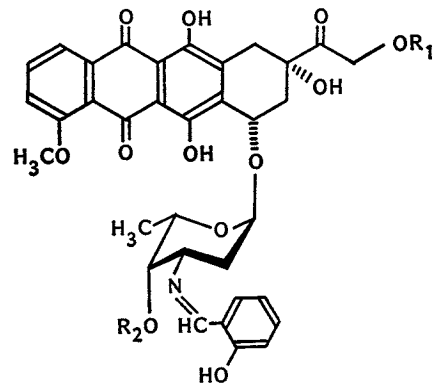
salicylaldehydes) in chloroform or in a mixture of chloroform and methanol gave the desired *N*-salicylidene derivatives (**7**~**12**) in moderate to good yields. Chemical shifts of SCHIFF base-methine protons of compounds **3** and **7**~**12** in their ^{1}H NMR spectra (400 MHz, $CDCl_3$) appeared at δ 7.96 for **10**, 8.18 for **8**, 8.22 for **11**, 8.26 for **9**, 8.27 for **7**, 8.31 for **12** and 8.35 for **3** in the order of decreasing inductive effect of aryl rings.

Electron-withdrawing groups (ester groups) were introduced into the aryl ring of salicylaldehyde (Scheme 1). We selected 3-hydroxy-4-(hydroxymethyl)benzoic acid[3] (**20**) as the pre-

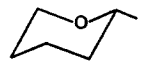


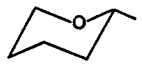
1 R=H

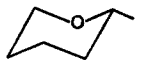
6 R= 

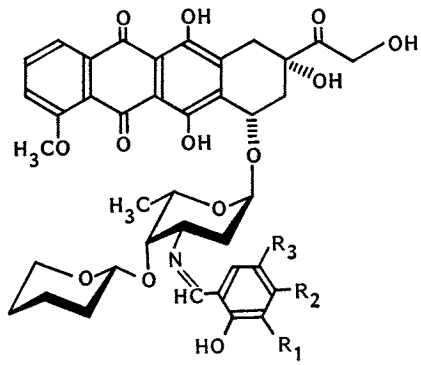


2 R_1=H R_2=H

3 R_1=H R_2= 

4 R_1=$CO(CH_2)_3CH_3$ R_2= 

5 R_1=$CO(CH_2)_8CH_3$ R_2= 



7 R_1=OH R_2=H R_3=H

8 R_1=H R_2=OH R_3=H

9 R_1=H R_2=H R_3=OH

10 R_1=OH R_2=OH R_3=H

11 R_1=H R_2=OCH_3 R_3=H

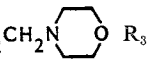
12 R_1=H R_2=H R_3=OCH_3

13 R_1=H R_2=$COOCH_3$ R_3=H

14 R_1=H R_2=$COOC_2H_5$ R_3=H

15 R_1=H R_2=$COOCH_2CF_3$ R_3=H

16 R_1=H R_2=$COOCH_2OCOC(CH_3)_3$ R_3=H

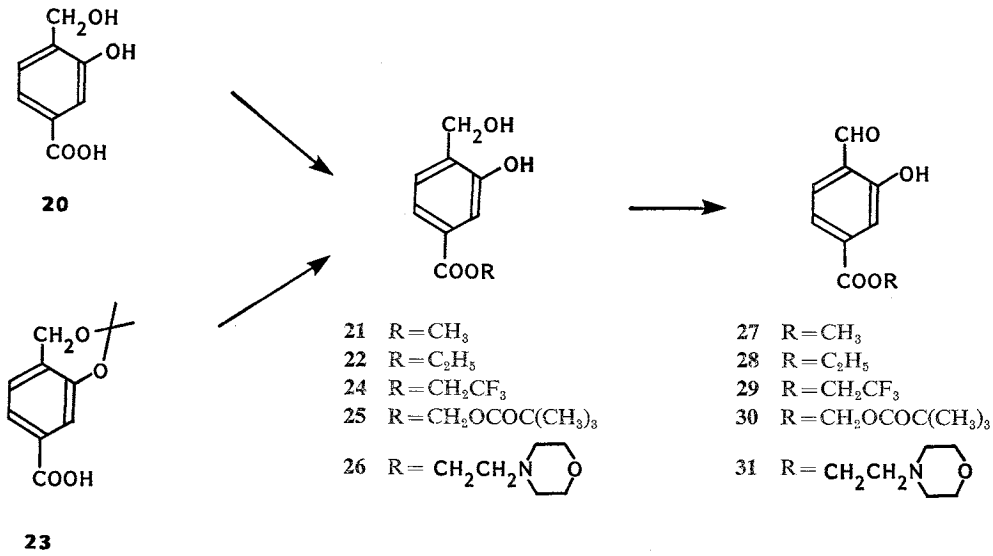
17 R_1=H R_2=  R_3=H

18 R_1=H R_2=$\overset{L}{C}HCOOCH_3$ (NHAc) R_3=H

19 R_1=H R_2=$\overset{L}{C}HCOOCH_3$ (NHBoc) R_3=H

Boc: *tert*-Butoxycarbonyl

Scheme 1.



cursor of 4-alkoxycarbonylsalicylaldehydes† (**27**~**31**). Compound **20** was known as a biological metabolite of forphenicinol[4] and also as an immunomodulator itself.[3] Esterification of **20** in anhydrous hydrogen chloride - methanol and - ethanol, followed by oxidation with active manganese dioxide gave 4-methoxycarbonyl- and 4-ethoxycarbonylsalicylaldehydes (**27** and **28**). *O*-Isopropylidene derivative[5] (**23**) of **20** was esterified with 2,2,2-trifluoroethanol in the presence of 4-dimethylaminopyridine and *N*,*N*'-dicyclohexylcarbodiimide in dichloromethane. After removal of the isopropylidene group with 90% trifluoroacetic acid, the hydroxymethyl group was oxidized with active manganese dioxide in ethyl acetate to give **29**. The sodium salt of **20** was treated with alkyl iodides (pivaloyloxymethyl and 2-morpholinoethyl iodides) in aqueous medium and then oxidized with active manganese dioxide to afford **30** and **31**.

Pirarubicin (**1**) was reacted with 4-alkoxycarbonylsalicylaldehydes (**27**~**31**) prepared above in chloroform to give stable products (**13**~**17**) in good yields. In ^{1}H NMR spectra of these compounds (**13**~**17**), the methine proton of the SCHIFF base moiety resonated at δ 8.42 to 8.45.

Forphenicine[6,7] (**32**), an inhibitor of alkaline phosphatase and an immunostimulator, is structurally similar to salicylaldehyde, that is, *o*-hydroxybenzaldehyde function. Since the direct coupling of **1** with **32** in dimethyl sulfoxide gave complicated results, *N*-acylforphenicine esters (**34** and **35**) were employed as aldehyde reagents. Compounds **34** and **35** were prepared from *N*-*tert*-butoxycarbonylforphenicinol[8] (**33**) (Scheme 2). The yield on *N*-acetylation of forphenicinol was poor because of the low solubility of the mother compound. Esterification of **33** with methyl iodide, deprotection of amino group followed by *N*-acetylation gave *N*-acetylforphenicinol methyl ester in good yield. It was subsequently oxidized to afford **34**. The methyl ester of **33** was oxidized by a similar method to yield **35**. Compounds **18** and **19** were synthesized by reaction of **1** with **34** and **35**, respectively.

Biological Evaluation

Compounds **1**~**19** were tested *in vitro* for cytotoxicity against leukemia P388 cells. As shown

† All analogues of alkyl 4-formyl-3-hydroxybenzoate used here were named as the derivatives of 4-alkoxycarbonylsalicylaldehyde.

Scheme 2.

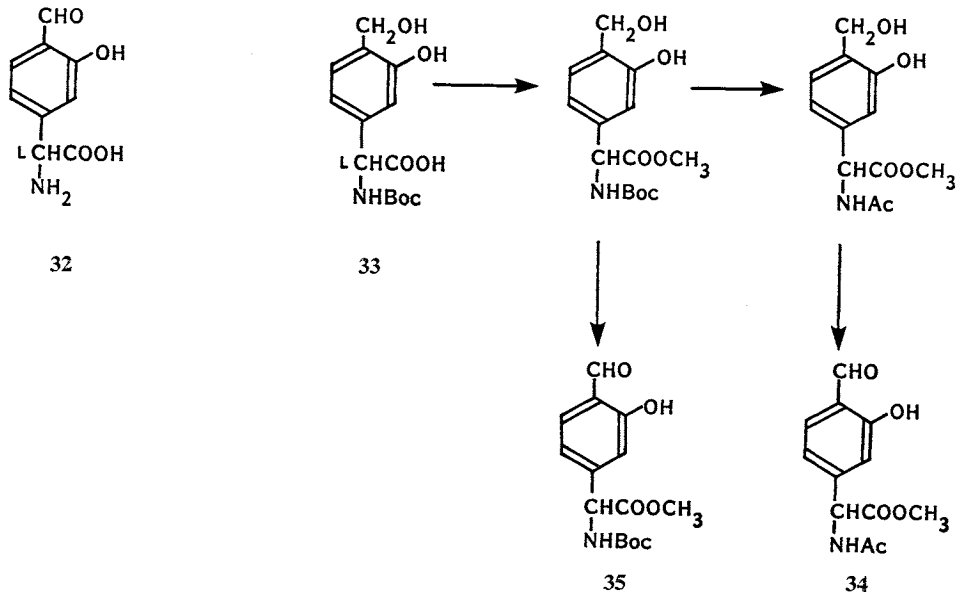


Fig. 1. Correlation between *in vivo* antitumor effects and Rf values.

50 mg/kg×9, po. Silica gel TLC: $CHCl_3$ - EtOH (20:1). R=0.781.

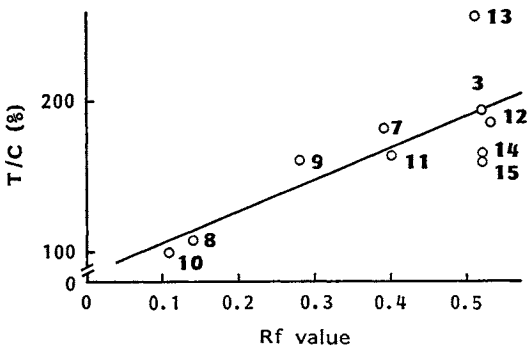


Table 1. Cytotoxicity against leukemia P388 cells.

Compounds	IC_{50} (ng/ml)
1	8.3
2	18.0
3	1.9
4	7.5
5	47.0
6	24.0
7	1.5
8	1.9
9	0.79
10	9.5
11	3.7
12	1.6
13	1.3
14	1.6
15	0.78
16	0.63
17	3.0
18	7.3
19	2.2

in Table 1, most of *N*-salicylidene derivatives of **1** had stronger cytotoxicity than **1**. Of the nineteen compounds evaluated, **9** (IC_{50} 0.79 ng/ml), **15** (0.78 ng/ml) and **16** (0.63 ng/ml) strongly inhibited the growth of P388 cells. By salicylidenation of **1**, potency was greatly enhanced. However, we could not predict any relationships between *in vitro* IC_{50} values and *in vivo* antitumor activities of these derivatives.

In vivo antitumor activity of the compounds synthesized here were evaluated in the murine L1210 leukemia. All compounds were administered orally and intraperitoneally into tumor-bearing mice. The results of the oral administration are shown in Table 2. Although **1** had no activity by oral administration at 50 mg/kg/day, its *N*-salicylidene derivative (**3**) showed good antitumor activity at dosages

Table 2. *In vivo* antitumor activity (T/C, %) against murine leukemia L1210 (po).

mg/kg/day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
50	116	114	194	120			181[a]	107	160	99	163	186	256	164[a]	161	148	160	135	109
25	103	90	157	113	108	110	157	99	111	99	113	180	153	152[a]	116	111	129	104	97
12.5	103	90	116	107	102		102	99	105	99	100	113	136	176[a]	110	117	105	99	97

[a] Loss of body weight of animals (>2 g) was observed.

Table 3. *In vivo* antitumor activity (T/C, %) against murine leukemia L1210 (ip).

mg/kg/day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
10	80[a]	84[a]	128[a]	157[a]	205	192	96[a]	101[a]	123[a]	95[a]	63[a]	>467[a]	142[a]	133[a]	91[a]	131[a]	131[a]	130[a]	139[a]
5	103[a]	108[a]	>657	205	241		>723	>462[a]	>741	>462[a]	>456[a]	214	>741	230[a]	218[a]	>686	>446	>623	248[a]
2.5	>686	211[a]	289	229	223	134	>750	177	357	>506	169	197	>741	218	345	>463	343	>452	230
1.25	337	167	134	225	181		175	192	277	335	163	>507	296	212	176	314	>434	197	255
0.625	>491	173	161	144	200	105	188	114	235	139	113	232	296	212	194	309	274	277	158
0.313	383	133	108	144	131		138	114	185	158	100	219	179	158	133	171	126	226	172
0.156	251	107	102	113	113	99	131	114	120	146		110	109	139	115	145		219	135
0.078	126	120						114	120	127		123	133	121	121	127		148	129

[a] Loss of body weight of animals (>2 g) was observed.

of 50 and 25 mg/kg/day. Furthermore, some substituted-salicylidene derivatives (**7**, **12**, **13** and **14**) were as active as **3**. Particularly, 4-methoxycarbonylsalicylidene derivative (**13**) showed significant prolongation of survival period with the broadest dose-response range.

The correlation between *in vivo* antitumor activity (T/C, %) of the selected compounds (**3** and **7**~**15**) by oral administration (50 mg/kg/day) and their Rf values on silica gel TLC is shown in Fig. 1. It shows that the more polar compound is less active (R=0.781). In other words, the lipophilicity of compounds affects antitumor activity by oral administration, assuming that the polarity of a compound is equivalent to the lipophilicity. Furthermore, we found that their antitumor effects by oral administration were well associated with chemical shifts of SCHIFF base-methine protons in ^{1}H NMR spectra (R=0.743). A compound having a chemical shift of the methine proton at lower field exhibits higher activity. It seems likely that the acidity of the methine proton considered here influences the absorbability of the compounds. Regarding compounds **16**~**19**, we suppose that their absorbability is reduced by the bulkiness of the ester group on the aryl ring of the SCHIFF base moiety.

The antitumor activity of compounds **1**~**19** by intraperitoneal injection into mice is shown in Table 3. Compound **3** exhibited a good antitumor activity. The activity of **6** was inferior to that of **3**. This suggested that the reduction of the imino group of **3** markedly diminished the antitumor activity. The 14-*O*-acylation of **3** (*i.e.* **4** and **5**) did not improve the activity. Among the substituted-salicylidene derivatives (**7**~**19**) tested, **7**, **13** and **18** showed excellent prolongation of survival period in tumor-bearing mice. Especially, **18** had the broadest dose range (0.078~5 mg/kg/day) of therapeutic effect. Interestingly, **18** contains the forphenicine residue in its structure.

Further biological evaluations of **13** and **18** are under way as potentially useful agents for cancer chemotherapy.

Experimental

General Methods

MP's were determined with a Yanagimoto micro melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter in concentration of 0.01 or 0.02% in chloroform or methanol at ambient temperature for derivatives of **1**. Mass spectra were obtained on a Hitachi M-80H mass spectrometer. ^{1}H NMR spectra were measured with a Varian EM-390 NMR spectrometer for 90 MHz or a Jeol JNM-GX 400 for 400 MHz in $CDCl_3$ unless otherwise noted. TLC was performed on silica gel (Kieselgel 60 F_{254}, Merck) developed with a mixture of chloroform - ethanol (20 : 1) and Rf values were calculated.

Satisfactory elemental analyses were obtained for all derivatives of **1**.

Antitumor Activity *In Vitro*

P388 leukemia cells, maintained on RPMI 1640 medium containing 10% fetal bovine serum and 10 μM of 2-hydroxyethyl disulfide, were incubated with target compounds for 72 hours. The number of viable cells was measured by the MTT assay[9,10] and IC_{50} values were calculated.

Antitumor Activity *In Vivo*

L1210 leukemia cells (10^5) maintained by serial intraperitoneal passage in our institute were implanted intraperitoneally into female CDF_1 mice (20±1 g) on day 0 and compounds were administered once daily on day 1~9. The survival period was observed for 60 days. T/C (%) was calculated from the median survival period of the treated group (T) of mice and that of the control group (C, survival period: 7~9 days). T/C values over 125% were judged to be active.

N-Salicylidene Derivative 3

Pirarubicin (**1**, 200 mg) was dissolved in chloroform (4 ml) and methanol (4 ml), and salicylalde-

hyde (54 mg) was added to the solution. The reaction solution was allowed to stand for 30 minutes and concentrated to give a dark red solid. The obtained solid was purified on a column of silica gel with a mixture of chloroform, ethanol and triethylamine (500:10:1) to afford **3** (195 mg) in 84% yield: MP 155~161°C (dec); $[\alpha]_D$ +380°; field desorption (FD)-MS *m/z* 731 (M^+); Rf 0.52; ^{1}H NMR δ 1.39 (3H, d, 6′-H), 2.18 (1H, dd, 8-H_{ax}), 2.4~2.5 (2H, m, 8-H_{eq}, 2′-H_{ax}), 3.09 (1H, d, 10-H_{ax}), 3.31 (1H, br d, 10-H_{eq}), 3.41 (1H, m, 6″-H), 3.62 (1H, m, 3′-H), 3.82 (1H, br s, 4′-H), 3.96 (1H, m, 6″-H), 4.08 (3H, s, OCH_3), 4.16 (1H, q, 5′-H), 4.59 (1H, br d, 2″-H), 4.79 (2H, d, 14-H), 5.37 (1H, br s, 7-H), 5.64 (1H, br d, 1′-H), 6.85, 6.94, 7.19 and 7.30 (salicylidene ring protons), 7.39 (1H, d, 3-H), 7.79 (1H, t, 2-H), 8.04 (1H, br d, 1-H), 8.35 (1H, s, N=CH).

14-*O*-Decanoate of **3** (**5**)

Compound **3** (399 mg) was dissolved in dry pyridine (13.5 ml) and the resultant solution was cooled at −15°C. To the chilled solution was added decanoyl chloride (207 mg) by portions for 6 hours and the solution was kept at −15°C overnight. After addition of methanol (0.2 ml), the mixture was concentrated to give a residue. This was dissolved in ethyl acetate (50 ml) and washed with aqueous sodium hydrogen carbonate and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated to afford a crude product. The product was re-salicylidenated with salicylaldehyde by the procedure mentioned above. Compound **5** (280 mg) was obtained in 58% yield: No definite melting point; $[\alpha]_D$ +280°; FD-MS *m/z* 885 (M^+); Rf 0.74; ^{1}H NMR δ 0.88 (3H, br t, CH_2CH_3), 1.41 (3H, d, 6′-H), 2.48 (2H, t, $COCH_2CH_2$), 2.96 (1H, d, 10-H_{ax}), 3.30 (1H, d, 10-H_{eq}), 4.06 (3H, s, OCH_3), 4.24 (1H, q, 5′-H), 4.62 (1H, br s, 2″-H), 5.11 and 5.38 (2H, each d, 14-H), 5.35 (1H, br s, 7-H), 5.64 (1H, br d, 1′-H), 7.78 (1H, t, 2-H), 8.03 (1H, d, 1-H), 8.38 (1H, s, N=CH).

14-*O*-Valerate of **3** (**4**)

Compound **4** was prepared from **3** and valeryl chloride by a similar method to **5**.

MP 124~128°C (dec); $[\alpha]_D$ +350°; FD-MS *m/z* 815 (M^+); Rf 0.71; ^{1}H NMR δ 0.94 (3H, t, CH_2CH_3), 1.41 (3H, d, 6′-H), 2.48 (2H, t, $COCH_2CH_2$), 2.90 (1H, d, 10-H_{ax}), 3.26 (1H, d, 10-H_{eq}), 4.03 (3H, s, OCH_3), 4.22 (1H, q, 5′-H), 4.62 (1H, br s, 2″-H), 5.11 and 5.38 (2H, each d, 14-H), 5.3 (1H, 7-H), 5.63 (1H, br d, 1′-H), 7.74 (1H, t, 2-H), 7.99 (1H, d, 1-H), 8.38 (1H, s, N=CH).

N-(2-Hydroxybenzyl) Derivative **6**

To a chilled solution of **3** (75 mg), acetic acid (8.2 μl) and salicylaldehyde (15 mg) in chloroform (1.1 ml) and methanol (1.1 ml) was added $NaB(CN)H_3$ (6.9 mg). The mixture was stirred at room temperature for 3.5 hours. After addition of acetone (0.5 ml), the solution was concentrated to give a residue. The solid was purified on preparative TLC with chloroform - ethanol (10:1) to yield pure **6** (14 mg, 16% yield): MP 124~135°C (dec); $[\alpha]_D$ +290°; secondary ion (SI)-MS *m/z* 734 (MH^+); Rf 0.42; ^{1}H NMR δ 1.35 (3H, d, 6′-H), 2.14 (1H, dd, 8-H_{ax}), 2.35 (1H, br d, 8-H_{eq}), 2.89 (1H, m, 3′-H), 3.04 (1H, d, 10-H_{ax}), 3.27 (1H, br d, 10-H_{eq}), 3.49 (1H, m, 6″-H), 3.87 (1H, br s, 4′-H), 3.93 (2H, ABq, $NHCH_2$), 4.00 (2H, m, 5′-H, 6″-H), 4.10 (3H, s, OCH_3), 4.60 (1H, br d, 2″-H), 4.72 (2H, ABq, 14-H), 5.32 (1H, br s, 7-H), 5.57 (1H, br d, 1′-H), 6.73, 6.81, 6.92 and 7.14 (aryl protons), 7.41 (1H, d, 3-H), 7.79 (1H, t, 2-H), 8.05 (1H, d, 1-H).

N-(3-Hydroxysalicylidene) Derivative **7**

Reaction of **1** with 3-hydroxysalicylaldehyde gave **7** in 97% yield by a similar procedure to **3**.

MP 161~167°C (dec); $[\alpha]_D$ +270°; FD-MS *m/z* 747 (M^+); Rf 0.39; ^{1}H NMR δ 1.39 (3H, d, 6′-H), 2.19 (1H, dd, 8-H_{ax}), 2.4~2.5 (2H, m, 8-H_{eq}, 2′-H_{ax}), 3.08 (1H, d, 10-H_{ax}), 3.31 (1H, br d, 10-H_{eq}), 3.40 (1H, m, 6″-H), 3.67 (1H, m, 3′-H), 3.83 (1H, br s, 4′-H), 3.96 (1H, m, 6″-H), 4.08 (3H, s, OCH_3), 4.16 (1H, q, 5′-H), 4.54 (1H, br d, 2″-H), 4.78 (2H, s, 14-H), 5.36 (1H, br s, 7-H), 5.65 (1H, br d, 1′-H), 6.69, 6.74 and 6.95 (salicylidene ring protons), 7.39 (1H, d, 3-H), 7.79 (1H, t, 2-H), 8.03 (1H, br d, 1-H), 8.27 (1H, s, N=CH).

N-(4-Hydroxysalicylidene) Derivative **8**

Reaction of **1** with 4-hydroxysalicylaldehyde gave **8** in 65% yield by a similar procedure to **3**.

MP 190~194°C (dec); $[\alpha]_D$ +330°; FD-MS *m/z* 748 (MH^+); Rf 0.14; ^{1}H NMR δ 1.38 (3H, d,

6′-H), 2.18 (1H, dd, 8-H_{ax}), 2.3~2.4 (2H, m, 8-H_{eq}, 2′-H_{ax}), 3.08 (1H, d, 10-H_{ax}), 3.30 (1H, br d, 10-H_{eq}), 3.41 (1H, m, 6″-H), 3.57 (1H, m, 3′-H), 3.79 (1H, br s, 4′-H), 3.95 (1H, m, 6″-H), 4.07 (3H, s, OCH_3), 4.14 (1H, q, 5′-H), 4.59 (1H, br d, 2″-H), 4.78 (2H, s, 14-H), 5.36 (1H, br s, 7-H), 5.63 (1H, br d, 1′-H), 6.30 (1H, dd, 5‴-H), 6.34 (1H, d, 3‴-H), 7.02 (1H, d, 6‴-H), 7.39 (1H, d, 3-H), 7.78 (1H, t, 2-H), 8.03 (1H, d, 1-H), 8.18 (1H, s, N=CH).

N-(5-Hydroxysalicylidene) Derivative **9**

Reaction of **1** with 5-hydroxysalicylaldehyde gave **9** in 87% yield by a similar procedure to **3**.

MP 161~163°C (dec); $[\alpha]_D$ +360°; FD-MS *m/z* 748 (MH^+); Rf 0.28; ^1H NMR δ 1.39 (3H, d, 6′-H), 2.18 (1H, dd, 8-H_{ax}), 2.4~2.5 (2H, m, 8-H_{eq}, 2′-H_{ax}), 3.08 (1H, d, 10-H_{ax}), 3.30 (1H, br d, 10-H_{eq}), 3.42 (1H, m, 6″-H), 3.62 (1H, m, 3′-H), 3.81 (1H, br s, 4′-H), 3.96 (1H, m, 6″-H), 4.07 (3H, s, OCH_3), 4.15 (1H, q, 5′-H), 4.59 (1H, br d, 2″-H), 4.78 (2H, d, 14-H), 5.36 (1H, br s, 7-H), 5.63 (1H, br d, 1′-H), 6.68 and 6.83 (salicylidene ring protons), 7.39 (1H, d, 3-H), 7.78 (1H, t, 2-H), 8.03 (1H, br d, 1-H), 8.26 (1H, s, N=CH).

N-(3,4-Dihydroxysalicylidene) Derivative **10**

Reaction of **1** with 3,4-dihydroxysalicylaldehyde gave **10** in a quantitative yield by a similar procedure to **3**.

MP 160~170°C (dec); $[\alpha]_D$ +310°; FD-MS *m/z* 764 (MH^+); Rf 0.11; ^1H NMR δ 1.38 (3H, d, 6′-H), 2.19 (1H, dd, 8-H_{ax}), 2.3~2.4 (2H, m, 8-H_{eq}, 2′-H_{ax}), 3.07 (1H, d, 10-H_{ax}), 3.30 (1H, br d, 10-H_{eq}), 3.41 (1H, m, 6″-H), 3.67 (1H, m, 3′-H), 3.83 (1H, br s, 4′-H), 3.96 (1H, m, 6″-H), 4.08 (3H, s, OCH_3), 4.14 (1H, q, 5′-H), 4.53 (1H, br d, 2″-H), 4.78 (2H, s, 14-H), 5.35 (1H, br s, 7-H), 5.63 (1H, br d, 1′-H), 6.31 and 6.59 (salicylidene ring protons), 7.39 (1H, d, 3-H), 7.78 (1H, t, 2-H), 7.96 (1H, s, N=CH), 8.03 (1H, d, 1-H).

N-(4-Methoxysalicylidene) Derivative **11**

Reaction of **1** with 4-methoxysalicylaldehyde gave **11** in 82% yield by a similar procedure to **3**.

MP 156~160°C (dec); $[\alpha]_D$ +340°; FD-MS *m/z* 762 (MH^+); Rf 0.40; ^1H NMR δ 1.38 (3H, d, 6′-H), 2.18 (1H, dd, 8-H_{ax}), 2.4~2.5 (2H, m, 8-H_{eq}, 2′-H_{ax}), 3.09 (1H, d, 10-H_{ax}), 3.31 (1H, br d, 10-H_{eq}), 3.41 (1H, m, 6″-H), 3.57 (1H, m, 3′-H), 3.79 (4H, 4′-H, 4‴-OCH_3), 3.96 (1H, m, 6″-H), 4.08 (3H, s, 4-OCH_3), 4.14 (1H, q, 5′-H), 4.59 (1H, br d, 2″-H), 4.78 (2H, d, 14-H), 5.37 (1H, br s, 7-H), 5.63 (1H, br d, 1′-H), 6.39 (1H, dd, 5‴-H), 6.43 (1H, d, 3‴-H), 7.06 (1H, d, 6‴-H), 7.39 (1H, d, 3-H), 7.79 (1H, t, 2-H), 8.04 (1H, br d, 1-H), 8.22 (1H, s, N=CH).

N-(5-Methoxysalicylidene) Derivative **12**

Reaction of **1** with 5-methoxysalicylaldehyde gave **12** in 47% yield by a similar procedure to **3**.

MP 139~148°C (dec); $[\alpha]_D$ +390°; FD-MS *m/z* 761 (M^+); Rf 0.53; ^1H NMR δ 1.39 (3H, d, 6′-H), 2.19 (1H, dd, 8-H_{ax}), 2.4~2.5 (2H, m, 8-H_{eq}, 2′-H_{ax}), 3.09 (1H, d, 10-H_{ax}), 3.31 (1H, br d, 10-H_{eq}), 3.41 (1H, m, 6″-H), 3.62 (1H, m, 3′-H), 3.74 (3H, s, 5‴-OCH_3), 3.82 (1H, br s, 4′-H), 3.96 (1H, m, 6″-H), 4.08 (3H, s, 4-OCH_3), 4.15 (1H, q, 5′-H), 4.59 (1H, br d, 2″-H), 4.78 (2H, d, 14-H), 5.37 (1H, br s, 7-H), 5.64 (1H, br d, 1′-H), 6.72 (1H, d, 6‴-H), 6.88 (1H, d, 3‴-H), 6.92 (1H, dd, 4‴-H), 7.39 (1H, d, 3-H), 7.79 (1H, t, 2-H), 8.04 (1H, br d, 1-H), 8.31 (1H, s, N=CH).

N-(4-(Methoxycarbonyl)salicylidene) Derivative **13**

Reaction of **1** with 4-(methoxycarbonyl)salicylaldehyde (**27**) gave **13** in 85% yield by a similar procedure to **3**.

MP 148~156°C (dec); $[\alpha]_D$ +350°; FD-MS *m/z* 790 (MH^+); Rf 0.51; ^1H NMR δ 1.40 (3H, d, 6′-H), 2.98 (1H, d, 10-H_{ax}), 3.30 (1H, d, 10-H_{eq}), 3.91 (3H, s, $COOCH_3$), 4.07 (3H, s, 4-OCH_3), 4.57 (1H, br s, 2″-H), 4.79 (2H, s, 14-H), 5.35 (1H, br s, 7-H), 5.63 (1H, br d, 1′-H), 7.79 (1H, t, 2-H), 8.03 (1H, dd, 1-H), 8.43 (1H, s, N=CH).

N-(4-(Ethoxycarbonyl)salicylidene) Derivative **14**

Reaction of **1** with 4-(ethoxycarbonyl)salicylaldehyde (**28**) gave **14** in 75% yield by a similar procedure to **3**.

MP 151~161°C (dec); $[\alpha]_D$ +310°; FD-MS *m/z* 803 (M^+); Rf 0.52; ^1H NMR δ 1.36 (3H, t,

CH_2CH_3), 1.38 (3H, d, 6′-H), 2.99 (1H, d, 10-H_{ax}), 3.32 (1H, d, 10-H_{eq}), 4.07 (3H, s, OCH_3), 4.36 (2H, q, $COOCH_2$), 4.57 (1H, br s, 2″-H), 4.78 (2H, br d, 14-H), 5.34 (1H, br s, 7-H), 5.63 (1H, br d, 1′-H), 7.78 (1H, t, 2-H), 8.04 (1H, d, 1-H), 8.43 (1H, s, N=CH).

N-(4-(2,2,2-Trifluoroethoxycarbonyl)salicylidene) Derivative **15**

Reaction of **1** with 4-(2,2,2-trifluoroethoxycarbonyl)salicylaldehyde (**29**) gave **15** in 92% yield by a similar procedure to **3**.

MP 141~147°C (dec); $[\alpha]_D$ +290°; SI-MS *m/z* 858 (MH^+); Rf 0.52; ^1H NMR δ 1.39 (3H, d, 6′-H), 3.08 (1H, d, 10-H_{ax}), 3.31 (1H, br d, 10-H_{eq}), 4.08 (3H, s, OCH_3), 4.55 (1H, br d, 2″-H), 4.68 (2H, q, $COOCH_2$), 4.78 (2H, s, 14-H), 5.37 (1H, br s, 7-H), 5.65 (1H, br d, 1′-H), 7.79 (1H, t, 2-H), 8.04 (1H, d, 1-H), 8.42 (1H, s, N=CH).

N-(4-(Pivaloyloxymethoxycarbonyl)salicylidene) Derivative **16**

Reaction of **1** with 4-(pivaloyloxymethoxycarbonyl)salicylaldehyde (**30**) gave **16** in 89% yield by a similar procedure to **3**.

MP 118~158°C (dec); $[\alpha]_D$ +300°; FD-MS *m/z* 889 (M^+); Rf 0.54; ^1H NMR δ 1.20 (9H, s, $C(CH_3)_3$), 1.39 (3H, d, 6′-H), 2.97 (1H, d, 10-H_{ax}), 3.29 (1H, d, 10-H_{eq}), 4.06 (3H, s, OCH_3), 4.55 (1H, br s, 2″-H), 4.8 (2H, 14-H), 5.34 (1H, br s, 7-H), 5.63 (1H, br s, 1′-H), 5.97 (2H, s, $COOCH_2O$), 7.79 (1H, t, 2-H), 8.03 (1H, d, 1-H), 8.44 (1H, s, N=CH).

N-(4-(2-Morpholinoethoxycarbonyl)salicylidene) Derivative **17**

Reaction of **1** with 4-(2-morpholinoethoxycarbonyl)salicylaldehyde (**31**) gave **17** in 90% yield by a similar procedure to **3**.

MP 124~136°C (dec); $[\alpha]_D$ +300°; FD-MS *m/z* 889 (MH^+); Rf 0.26; ^1H NMR δ 1.39 (3H, d, 6′-H), 2.55 (4H, br t, $N(CH_2CH_2)_2O$), 2.75 (2H, t, $COOCH_2CH_2$), 3.71 (4H, br t, $N(CH_2CH_2)_2O$), 4.07 (3H, s, OCH_3), 4.45 (2H, t, $COOCH_2$), 4.8 (2H, 14-H), 5.33 (1H, br s, 7-H), 5.63 (1H, br s, 1′-H), 7.78 (1H, t, 2-H), 8.02 (1H, d, 1-H), 8.45 (1H, s, N=CH).

Compound **18**

Reaction of **1** with *N*-acetylforphenicine methyl ester (**34**) gave **18** in 63% yield by a similar procedure to **3**.

MP 158~171°C (dec); $[\alpha]_D$ +370°; FD-MS *m/z* 861 (MH^+); Rf 0.29; ^1H NMR δ 1.38 (3H, d, 6′-H), 2.03 (3H, s, $COCH_3$), 2.97 (1H, d, 10-H_{ax}), 3.29 (1H, d, 10-H_{eq}), 3.73 (3H, s, $COOCH_3$), 4.07 (3H, s, 4-OCH_3), 4.58 (1H, br s, 2″-H), 4.78 (2H, br s, 14-H), 5.33 (1H, br s, 7-H), 5.55 (1H, d, α-H), 5.6 (1H, 1′-H), 7.79 (1H, t, 2-H), 8.03 (1H, d, 1-H), 8.37 (1H, s, N=CH).

Compound **19**

Reaction of **1** with *N-tert*-butoxycarbonylforphenicine methyl ester (**35**) gave **19** in 99% yield by a similar procedure to **3**.

MP 155~161°C (dec); $[\alpha]_D$ +320°; FD-MS *m/z* 918 (M^+); Rf 0.51; ^1H NMR δ 1.39 (9H, s, $C(CH_3)_3$), 2.83 (1H, d, 10-H_{ax}), 3.17 (1H, d, 10-H_{eq}), 3.70 (3H, s, $COOCH_3$), 4.03 (3H, s, 4-OCH_3), 4.60 (1H, br s, 2″-H), 4.78 (2H, s, 14-H), 5.1~5.4 (2H, br s, 7-H, α-H), 5.60 (1H, br s, 1′-H), 7.73 (1H, t, 2-H), 7.92 (1H, d, 1-H), 8.38 (1H, s, N=CH).

4-(Methoxycarbonyl)salicylaldehyde (**27**)

To a solution of methyl 3-hydroxy-4-hydroxymethylbenzoate[8] (**21**, 298 mg) in ethyl acetate (30 ml), active manganese dioxide (1.5 g) was added and the mixture was stirred at room temperature for 1 hour. The solid was removed by filtration and washed with methanol (15 ml×4). The combined filtrate was concentrated to give a solid which was purified on silica gel column chromatography (chloroform - hexane, 2:1) to afford a colorless solid of **27** (153 mg) in 52% yield: MP 131~134°C; electron impact (EI)-MS *m/z* 180 (M^+); ^1H NMR δ 3.96 (3H, s, CH_3), 7.69 (3H, s, Ar), 10.05 (1H, s, CHO).

4-(Ethoxycarbonyl)salicylaldehyde (**28**)

Ethyl 3-hydroxy-4-hydroxymethylbenzoate[8] (**22**, 412 mg) was oxidized by a similar procedure to

27 to give colorless needles of **28** (251 mg, 62%): MP 85~88°C; EI-MS *m/z* 194 (M^+); ^{1}H NMR δ 1.40 (3H, t, CH_3), 4.41 (2H, q, CH_2), 7.69 (3H, s, Ar), 10.03 (1H, s, CHO).

4-(2,2,2-Trifluoroethoxycarbonyl)salicylaldehyde (**29**)

A mixture of *O*-isopropylidene 3-hydroxy-4-hydroxymethylbenzoic acid[5] (**23**, 981 mg), *N,N'*-dicyclohexylcarbodiimide (DCC) (1.167 g) and 4-dimethylaminopyridine (115 mg) in anhydrous dichloromethane (30 ml) was stirred at room temperature for 10 minutes. 2,2,2-Trifluoroethanol (0.41 ml) was added, and the mixture was stirred at room temperature for 1 hour. The precipitate was filtered off and was washed with chloroform (10 ml×2). The combined filtrate was concentrated to give a residue which was purified on silica gel column chromatography (chloroform - hexane, 1:1) to afford colorless plates of the 2,2,2-trifluoroethyl ester (1.333 g, 97%): MP 44°C; EI-MS *m/z* 290 (M^+); ^{1}H NMR δ 1.55 (6H, s, $C(CH_3)_2$), 4.69 (2H, q, $COOCH_2$), 4.90 (2H, s, $ArCH_2$), 7.09 (1H, d, 5-H), 7.57 (1H, br s, 2-H), 7.64 (1H, dd, 6-H). To a solution of the ester (1.3 g) in methanol (100 ml), was added 90% aqueous TFA (13 ml), and the solution was kept at room temperature for 2 days. After the addition of triethylamine (5.0 ml), the solution was concentrated to give a residue which was extracted with chloroform (200 ml). The organic layer was washed with water (100 ml×2), dried over anhydrous sodium sulfate and concentrated to afford a colorless solid of diol ester **24** (923 mg): ^{1}H NMR δ 4.67 (2H, q, $COOCH_2$), 4.90 (2H, s, $ArCH_2$), 7.15 (1H, d, 5-H), 7.55 (1H, d, 2-H), 7.56 (1H, dd, 6-H). Compound **24** was oxidized with active manganese dioxide in a similar condition. Purification on silica gel column chromatography (chloroform - hexane, 2:3 to 3:2) to afford colorless needles of **29** (459 mg, 40% from **23**): MP 59°C; EI-MS *m/z* 249 (MH^+); ^{1}H NMR δ 4.73 (2H, q, $COOCH_2$), 7.73 (3H, s, Ar), 10.06 (1H, s, CHO).

4-(Pivaloyloxymethoxycarbonyl)salicylaldehyde (**30**)

To a solution of sodium salt of **20** (350 mg) in water (3.5 ml), THF (21 ml) and DMF (3.5 ml) was added iodomethyl pivalate (1.7 ml), the mixture was stirred at room temperature for 2 hours. Evaporation of solvent gave a residue which was extracted with chloroform (70 ml). The organic layer was washed with water (70 ml), dried over anhydrous sodium sulfate and concentrated to give a residue. The residue was purified on preparative silica gel TLC (chloroform - methanol, 10:1). Colorless needles of **25** was obtained (272 mg, 52%): MP 97~98°C; ^{1}H NMR δ 1.21 (9H, s, $C(CH_3)_3$), 4.92 (2H, s, $ArCH_2$), 5.98 (2H, s, $COOCH_2O$), 7.14 (1H, d, 5-H), 7.57 (1H, dd, 6-H), 7.6 (1H, 2-H). The ester **25** (218 mg) was oxidized by a similar procedure to give a colorless solid of **30** (140 mg, 65%): MP 78~80°C; EI-MS *m/z* 280 (M^+); ^{1}H NMR δ 1.23 (9H, s, $C(CH_3)_3$), 5.97 (2H, s, $COOCH_2O$), 7.71 (3H, s, Ar), 10.06 (1H, s, CHO).

4-(2-Morpholinoethoxycarbonyl)salicylaldehyde (**31**)

A mixture of sodium salt of **20** (236 mg) and 4-(2-iodoethyl)morpholine (600 mg, prepared from 4-(2-chloroethyl)morpholine and sodium iodide), in THF (12 ml) and water (2 ml) was kept at room temperature for 1 day, and was concentrated. To the residue, water (50 ml) and triethylamine (0.1 ml) were added, and the morpholinoethyl ester was extracted with chloroform (15 ml×5). The organic layer was dried over anhydrous sodium sulfate and concentrated to give a residue. The residue was purified on silica gel column chromatography (chloroform - ethanol, 15:1) to afford colorless plates of **26** (228 mg, 65%): MP 96~100°C; ^{1}H NMR δ 2.58 (4H, m, $N(CH_2CH_2)_2O$), 2.77 (2H, t, $COOCH_2CH_2$), 3.72 (4H, m, $N(CH_2CH_2)_2O$), 4.44 (2H, t, $COOCH_2$), 4.82 (2H, s, $ArCH_2$), 7.11 (1H, d, 5-H), 7.42 (1H, s 2-H), 7.47 (1H, br d, 6-H). This ester **26** (228 mg) was oxidized in similar conditions to give colorless needles of **31** (123 mg, 54%): MP 60°C; EI-MS *m/z* 279 (M^+); ^{1}H NMR δ 2.57 (4H, m, $N(CH_2CH_2)_2O$), 2.77 (2H, t, $COOCH_2CH_2$), 3.72 (4H, m, $N(CH_2CH_2)_2O$), 4.49 (2H, t, $COOCH_2$), 7.69 (3H, s, Ar), 10.06 (1H, s, CHO).

N-tert-Butoxycarbonylforphenicinol Methyl Ester

To a mixture of *N-tert*-butoxycarbonylforphenicinol sodium salt (prepared from **33**[6] (372 mg) and 1 M sodium hydroxide (1.25 ml)) in anhydrous DMF (7.5 ml), was added methyl iodide (0.16 ml) at 0°C, and the mixture was stirred at room temperature for 1 day. Concentration of the mixture gave a residue which was extracted with ethyl acetate (50 ml). The organic layer was washed with water

(25 ml×2), dried over anhydrous sodium sulfate and concentrated to give a syrup. This was purified on preparative silica gel TLC (chloroform - methanol, 10:1) to afford a colorless syrup of *N-tert*-butoxycarbonylforphenicinol methyl ester (354 mg, 91%): $[\alpha]_D$ +119° (*c* 1.0, chloroform); EI-MS *m/z* 311 (M^+); ^{1}H NMR δ 1.41 (9H, s, $C(CH_3)_3$), 3.69 (3H, s, $COOCH_3$), 4.71 (2H, s, $ArCH_2$), 5.18 (1H, br d, α-H), 6.80 (1H, d, 6-H), 6.84 (1H, s, 2-H), 7.04 (1H, d, 5-H).

N-Acetylforphenicine Methyl Ester (**34**)

A solution of *N-tert*-butoxycarbonylforphenicinol methyl ester (500 mg) (prepared above) in 90% aqueous TFA (5 ml) was kept at 0°C for 30 minutes. The solution was concentrated to give a colorless oil which was triturated with a mixture of isopropyl ether and ethyl ether (2:1, 25 ml). The precipitate was dried and dissolved in anhydrous methanol (25 ml). To the solution, anhydrous pyridine (0.26 ml) and acetic anhydride (0.23 ml) was added, and the solution was allowed to stand at room temperature for 1 day. After the addition of water (0.22 ml), the solution was concentrated to give an oil. The oil was dissolved in ethyl acetate (100 ml), washed with brine, dried over anhydrous sodium sulfate and concentrated to give a residue. This was purified on preparative silica gel TLC (chloroform - methanol, 10:1) to afford a colorless syrup of *N*-acetylforphenicinol methyl ester (253 mg, 62%): $[\alpha]_D$ +161° (*c* 1.0, methanol); EI-MS *m/z* 253 (M^+); ^{1}H NMR (CD_3OD) δ 2.02 (3H, s, $COCH_3$), 3.73 (3H, s, $COOCH_3$), 4.67 (2H, s, $ArCH_2$), 5.38 (1H, s, α-H), 6.8~6.9 (2H, m, 2-H, 6-H), 7.30 (1H, d, 5-H). *N*-Acetylforphenicinol methyl ester (180 mg) was oxidized in similar conditions to give a colorless syrup of **34** (110 mg, 62%): $[\alpha]_D$ +81° (*c* 1.0, chloroform); EI-MS *m/z* 251 (M^+); ^{1}H NMR δ 2.05 (3H, s, $COCH_3$), 3.76 (3H, s, $COOCH_3$), 5.62 (1H, d, α-H), 7.02 (1H, s, 2-H), 7.06 (1H, br d, 6-H), 7.58 (1H, d, 5-H), 9.94 (1H, s, CHO).

N-tert-Butoxycarbonylforphenicine Methyl Ester (**35**)

N-tert-Butoxycarbonylforphenicinol methyl ester (331 mg) was oxidized in similar conditions to give a colorless syrup of **35** (197 mg, 60%): $[\alpha]_D$ +134° (*c* 1.0, chloroform); EI-MS *m/z* 309 (M^+); ^{1}H NMR δ 1.41 (9H, s, $C(CH_3)_3$), 3.74 (3H, s, $COOCH_3$), 5.33 (1H, br d, α-H), 7.02 (1H, s, 2-H), 7.06 (1H, br d, 6-H), 7.58 (1H, d, 5-H), 9.93 (1H, s, CHO).

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