25. Colchicine Models: Synthesis and Antitubulin Activity of 2′-Monosubstituted and 2′,5-Disubstituted 2,3,4,4′-Tetramethoxy-1,1′-biphenyls. Synthesis of 4,4′,5′,6′-Tetramethoxy-1,1′-biphenyl-2,3′-dicarboxylic Acid

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This paper is dedicated to our friend and colleague Dr. *Tetsuji Kametani*, Hoshi College of Pharmacy, Tokyo, Japan, who died on October 11, 1988

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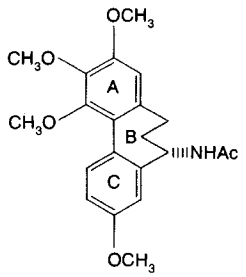
Reductive amination of 2,3,4,4′-tetramethoxybiphenyl-2-carbaldehyde[1]) (**4**) with $MeNH_2$ afforded methylamine **5** *(Scheme 1)*. Hydroxymethylation of amine **8**, prepared similarly from **4** by reductive amination with benzylamine followed by *N*-methylation, afforded alcohol **12** which was converted the 5-methyl-substituted methylamine **14** by conventional chemical reactions *(Scheme 2)*. Methylamine **14** was also obtained from ester **16** after hydroxymethylation to alcohol **17** and conventional manipulation of alcohol and ester functions *(Scheme 2)*. Both amines **5** and **14** as well as the 2′,5-dimethyl-substituted biphenyl **26** prepared from the dialdehyde **25** by a *Wolff-Kishner* reduction, did not show noteworthy activity in the tubulin binding assay or as inhibitors of tubulin polymerization *(Table)*. However, the 2′-ethyl-substituted biphenyl **11** prepared from **4** by reaction with MeLi followed by dehydration and catalytic reduction of styrene **10** *(Scheme 1)* showed appreciable activity in both assays, coming close to that of known phenyltropolone models. The X-ray analysis of **14**·HCl and **11** showed significant difference in the orientation of the rings with respect to one another *(Fig.)*.

Introduction. – *N*-Acetylcolchinol 3-*O*-methyl ether (**1**) is a member of the allo-series of colchicinoids with a benzenoid ring C instead of the seven-membered tropolonic ring C as present in colchicine [1]. Compound **1** which can be obtained from colchicine (**2** with Ac–NH instead of CH_3–NH) by treatment with H_2O_2 at elevated temperature [2], binds extremely well to tubulin *in vitro* [3] [4]. It serves, for this reason, as a model compound to study structural and conformational features of compounds related to colchicine binding to tubulin [4]. Our initial attempts to study biphenyls with antitubulin activity of compounds related to **1** led to tetramethoxybiphenyl **3**, which showed 20% of the binding affinity of colchicine, arresting mitosis in metaphase in a similar fashion but at *ca.* 10-fold higher concentration than the natural alkaloid. It seemed, therefore, of interest to prepare analogs of **3** with other substituents at C(2′)[1]) and analogs with additional substituents in

[1]) In order to easy the discussion, we use the same skeletal numbering for all biphenyls; systematic names are given in the *Exper. Part.*



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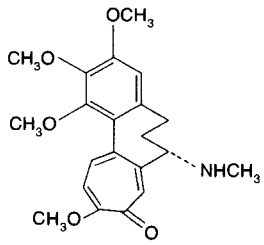

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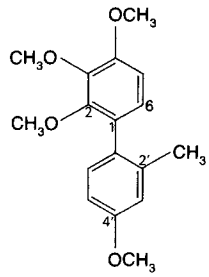

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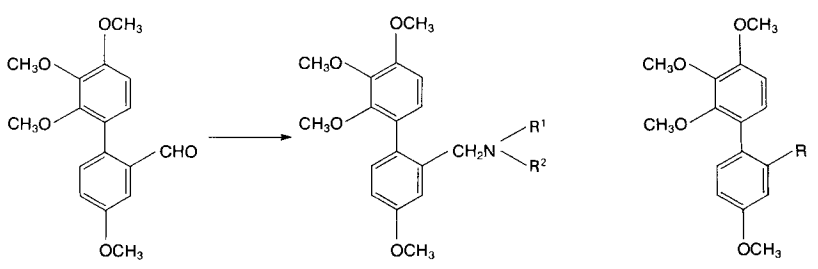
the other phenyl ring, hopefully at C(6), since such substitution would greatly influence atropisomerism now recognized to be important in colchicine's binding to tubulin [5].

Our first attempts were directed toward synthesis of biphenyls with a (methylamino)methyl group at C(2′)[1]), a subtitution present in the antitumor alkaloid demecolcine (**2**) [1], and compounds possessing an Et group at C(2′) instead of a Me group as present in **3**. Hydroxymethylation of the trimethoxy-substituted phenyl ring with aqueous formaldehyde was accomplished with amine **8** and ester **16**, and subsequent conventional chemical reactions led to 5-methyl-substituted amine **14**, thus opening a new route to otherwise almost inaccessible biphenyls. Several of the biphenyls prepared were examined for inhibitory effects on tubulin polymerization and the binding of radiolabelled colchicine to tubulin. Good activity in these assays is often associated with antitumor activity [6] and inhibition of amyloid migration, associated with drugs useful in Familial Mediterranean Fever [7].

Chemistry[2]). – On reaction with $MeNH_2$ in the presence of sodium cyanoborohydride, the known aldehyde **4** [4] afforded amine **5** which was transformed to acetamide **6**. Similarly, the amine **7** was prepared from **4** and benzylamine and converted into tertiary amine **8** by reductive *N*-methylation *(Scheme 1)*. Synthesis of 2′-ethyl-substituted biphenyl **11** from **4** was accomplished by reaction with MeLi [8], dehydration of alcohol **9** to vinyl-substituted biphenyl **10**, and catalytic reduction of the latter with Pd/C catalyst

Scheme 1



5 $R^1 = CH_3$, $R^2 = H$
6 $R^1 = CH_3$, $R^2 = COCH_3$
7 $R^1 = Bn$, $R^2 = H$
8 $R^1 = Bn$, $R^2 = CH_3$

9 R = CHOHCH$_3$
10 R = CH=CH$_2$
11 R = CH$_2$CH$_3$

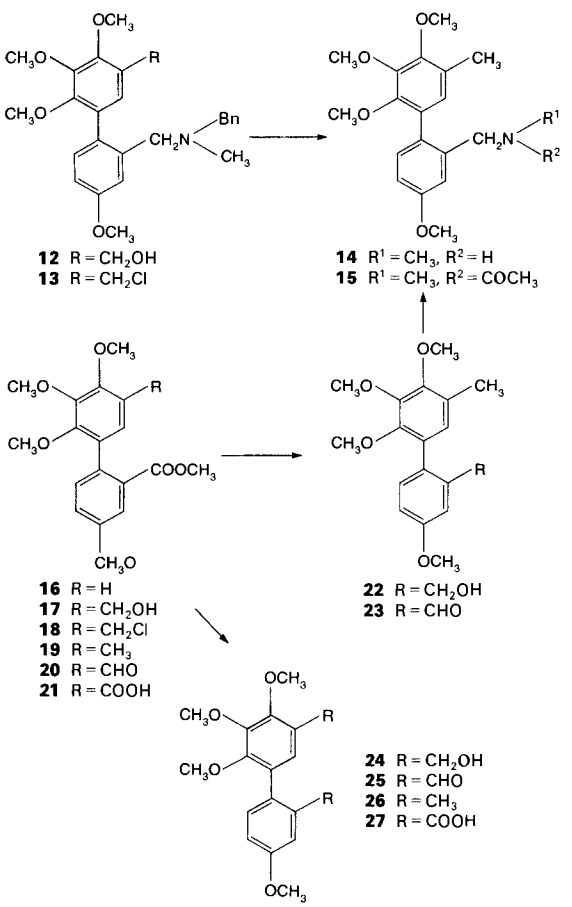
[2]) In solution, biphenyls are probably in an equilibrium with atropisomers. We show their structures by conventional presentations (*Schemes 1* and *2*).

(*Scheme 1;* ^{1}H-NMR of **11**: 4 MeO at 3.92, 3.90, 3.85, and 3.58 ppm, 5 arom. H at 7.09, 6.85, 6.83, 6.76, and 6.70 ppm, and Et at 2.48 and 1.08 ppm; X-ray analysis of **11**, see below).

Hydroxymethylation of amine **8** with formaldehyde in 1N HCl at 130° afforded alcohol **12** in 78.7% yield. Conversion of **12** into amine **14** was accomplished by reaction with $SOCl_2$ (→**13**) followed by catalytic reduction with Pd/C catalyst *(Scheme 2)*. The structure of **14** which was obtained as a crystalline hydrochloride was assigned on the basis of an X-ray analysis (see below) and its convertion to the amide **15**.

Hydroxymethylation could also be achieved with the known ester **16** [4], a useful intermediate to prepare amine **14** by an alternate route *(Scheme 2)*. Thus, alcohol **17** obtained from **16** afforded, after treatment with $SOCl_2$ (→**18**) and catalytic reduction with Pd/C catalyst the biphenyl **19**. Oxidation of **17** gave the derivatives **20** and **21**. Reduction of **19** with $LiAlH_4$ (→**22**), followed by oxidation with pyridinium chlorochromate yielded aldehyde **23**. Finally, reductive amination of **23** with $MeNH_2$ and sodium cyanoborohydride gave amine **14**, identical in every respect with material pre-

Scheme 2



pared from **12**. Thus hydroxymethylation of ester **16** took place at C(5), a position activated by the *o,p*-positioned MeO groups.

Preparation of the 2′,5-disubstituted biphenyl **26** was achieved from alcohol **17** by reduction with $LiAlH_4$ (→**24**), oxidation with pyridinium chlorochromate (→**25**), and *Wolff-Kishner* reduction to biphenyl **26** *(Scheme 2)*. The 2′,5-dicarboxylic acid **27** was obtained by *Jones* oxidation of alcohol **17** after alkaline hydrolysis of intermediate ester **21** (^{1}H-NMR **27**: 4 MeO groups at 4.17, 3.89, 3.86, and 3.66 ppm, 4 arom. H at 7.78, 7.53, 7.24, and 7.14 ppm).

X-Ray Analysis of 11 and 14·HCl. – For **11**, $C_{18}H_{22}O_4$, mol.wt. 302.36; monoclinic, space group C_2/c: $a = 16.547(3)$, $b = 8.185(1)$, $c = 25.433(4)$ Å, $\beta = 106.98(1)°$, $V = 3294.3(9)$ Å^3, $Z = 8$, $d_{calc.} = 1.22$ mg/mm^{-3}, $\mu = 0.66$ mm^{-1}. For **14**·HCl, $C_{19}H_{26}NO_4^+ \cdot Cl^-$, mol.wt. 367.87; triclinic, space group $P\bar{1}$; $a = 5.948(1)$, $b = 12.468(2)$, $c = 14.351(2)$ Å, $\alpha = 103.74(1)$, $\beta = 100.60(1)$, $\gamma = 100.41(1)°$, $V = 987.6(3)$ Å^3, $Z = 2$, $d_{calc.} = 1.24$ mg/mm^{-3}, $\mu = 1.91$ mm^{-1}. Measurements for both compounds were obtained with a *Nicolet R3m/v* automatic diffractometer using Cu$K\alpha$ radiation ($\lambda = 1.54178$ Å) with an incident-beam graphite monochromator [9]. The 2615 (2885 for **14**) independent reflections were measured at r.t. using the $\theta/2\theta$ scan technique with a variable scan rate out to $2\theta_{max} = 115°$ (110° for **14**). Data were corrected for *Lorentz* and polarization effects, but absorption effects were ignored. Both structures were solved by direct methods and refined by full-matrix least-squares techniques (non-H-atoms anisotropic; H-atoms riding on covalently bonded atoms with fixed isotropic thermal parameters except for the H-atoms on N(2) in **14** which were refined isotropically) using the 1701 (2503 for **14**) reflections for which $F_o > 3\sigma(F_o)$ to a final R factor of 0.065, $R_w = 0.062$ (for **14**, $R = 0.064$, $R_w e = 0.062$). The function minimized by the least-squares was $\Sigma w(|F_o| - F_c|)^2$ where $w = 1/[\sigma^2(|F_o|)^2 + g(F_o)^2]$ and $g = 0.00023$. The goodness of fit parameter was 1.69 (1.61 for **14**). All calculations were carried out with the SHELXTL system of programs [9].

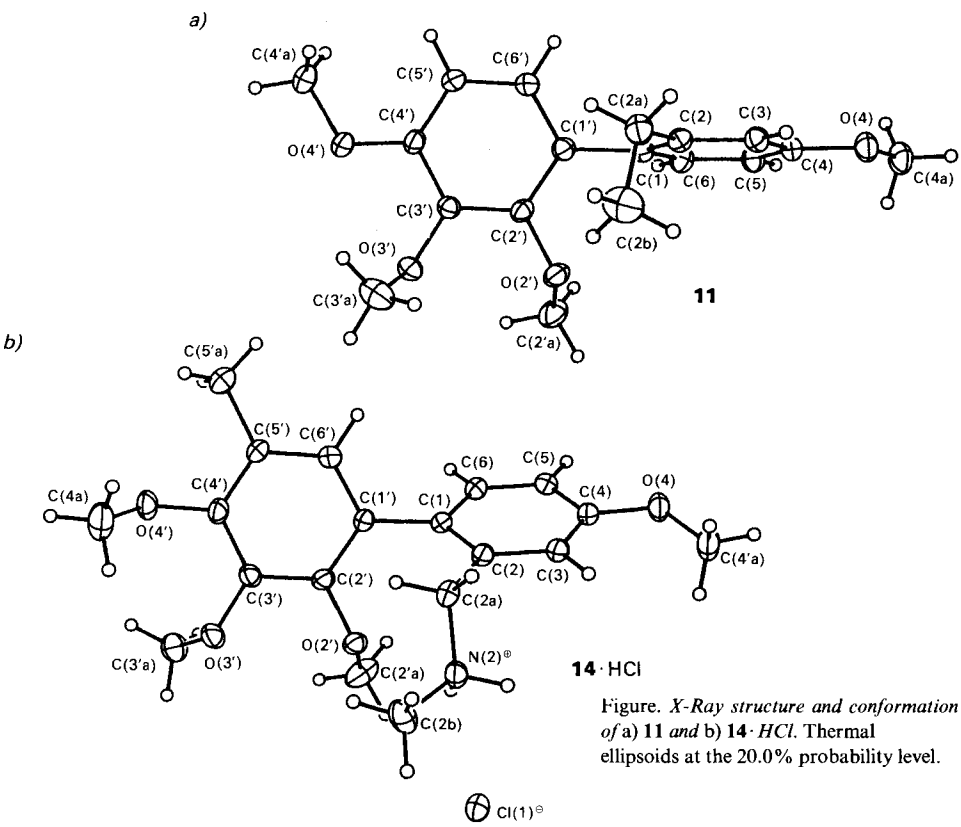


Figure. *X-Ray structure and conformation of* a) **11** *and* b) **14**·*HCl*. Thermal ellipsoids at the 20.0% probability level.

Discussion. – The *Figure* shows the results of the X-ray studies on both **11** and **14**·HCl. Tables of coordinates, bond lengths, and angles have been deposited with the *Cambridge Crystallographic Data Base* [10]. For **14**, the X-ray results show that the methylation took place at C(5′) rather than C(6′). The six-membered rings are planar in both molecules, but there is a significant difference in the orientation of the rings with respect to one another. In **11**, they are approximately perpendicular to one another (C(6′)–C(1′)–C(1)–C(6) torsion angle is −96.2°), while in **14**, they are *gauche* with respect to one another (C(6′)–C(1′)–C(1)–C(6) torsion = 53.7°). In addition, three of the four MeO substituents, common to both **11** and **14**, have differing conformations with respect to the phenyl rings. Torsion angles for the MeO groups are (for **11** and **14**, resp.): C(1′)–C(2′)–O(2′)–C(2′a) = −114.6, −100.0°; C(2′)–C(3′)–O(3′)–C(3′a) = −120.1, 97.0°; C(3′)–C(4′)–O(4′)–C(4a′) = 175.9, 72.4°; C(3)–C(4)–O(4)–C(4a) = 178.0, 5.0°. There are also differences in the intramolecular approaches between O(2′) and the substituents on C(2): in **11**, O(2′)···C(2a) = 4.00 and O(2′)···C(2b) = 3.73 Å, while in **14**, O(2′)···C(2a) = 2.94 and O(2′)···N(2) = 2.86 Å, both of which are close to *van der Waals* approaches. In **14**, there is one intermolecular N···Cl H-bond (N···Cl = 3.08 Å, H···Cl = 2.16 Å, N–H···Cl = 162.8°).

Biological Evaluation. – The compounds listed in the *Table* were first examined as potential inhibitors of the polymerization of purified tubulin *(Table, Exper. 1)*. They were compared to colchicine (**2** with AcNH instead of MeNH), *N*-acetylcolchinol 3-*O*-methyl ether (**1**), and the biphenyl **3** previously studied [4]. Only one of the new biphenyls,

Table. *Biological Properties of Biphenyls*

Agent	*Exper. 1* Tubulin polymerization[a])	*Exper. 2* Colchicine binding[b])	
		Inhibitor/colchicine	
		1:1	10:1
	IC_{50} [μM]	% inhibition	
N-acetylcolchinol 3-*O*-methyl ether (**1**)	1–2	89	100
Colchicine (**2**, AcNH instead of MeNH)	2–3	25	82
2′-Methyl-2,3,4,4′-tetramethoxybiphenyl (**3**)	10–15	37	81
5	> 100	5	10
6	> 100	0	34
8	> 100	0	7
11	7.5–10	34	82
14	> 100	2	2
15	> 100	4	8
20	> 75[c])	5	13
26	> 100	1	14

[a]) Reaction mixtures contained 1.0 μM monosodium glutamate (pH 6.6 with HCl), 1.0 mM $MgCl_2$, 0.4 mM GTP, 1.0 mg/ml (10 μM) tubulin, and various drug concentrations. All components were preincubated for 15 min at 37° prior to addition of GTP. The *IC* range describes concentrations at which less than and greater than 50% inhibition of polymerization after 20 min at 37° was reproducibly observed [12].

[b]) Reaction mixtures contained 0.1 mg/ml (1 μM) tubulin, 5 μM [*ring-A*-4-^{3}H]colchicine, and the indicated inhibitor at either 5 or 50 μM. Incubation was for 10 min at 37°. For further details, see [12].

[c]) Insoluble in H_2O at higher concentration.

i.e. **11**, had significant inhibitory effects in this assay, similar to that of *Fitzgeralds* phenyltropolone [4] [11]. Compound **1** and colchicine were still more inhibitory than **11**; but if **11** is a mixture of two atropisomers, where only one of the two binds to the protein, then the active isomer was nearly as effective as colchicine [5]. Essentially the same result was obtained when inhibition of the binding of radiolabelled colchicine to purified tubulin was examined *(Table, Exper. 2)*, with **11** and **3** being equipotent to colchicine (the relatively weak inhibition by low concentrations of non-radiolabelled colchicine might derive from the slow binding of the drug to tubulin and the use of a subsaturating amount of radiolabelled colchicine in the experiment).

Conclusions. – The hydroxymethylation realized with amine **8** and ester **16** occurs at the sterically least hindered atom C(5) activated by *ortho-* and *para-*positioned MeOH groups. This allows, as shown, an entry into a wide variety of 2′,5-disubstituted 2,3,4,4′-tetramethoxybiphenyls.

It can be seen from inspection of *Dreiding* models and the X-ray crystal structure of **14**·HCl that the two phenyl rings are not coplanar, with equilibria of isomers controlled by the substituent at C(2′). The inactivity of biphenyl **26**, when compared with the good activity of biphenyl **3** in the tubulin binding assay, can be explained by the presence of Me–C(5) in **26**. Although inactivity in binding to tubulin of amine **14** can be argued on this molecular variation, the inactivity of amine **5** which lacks Me–C(5) cannot be explained with this argument and requires further comment. The X-ray structure of **14**·HCl shows the aromatic rings *gauche* to each other and the aminomethyl group H-bonded with MeO–C(2). It has to be assumed that a similar situation, responsible for inactivity, would exist for the amine **5**, since the binding assay is carried out under slightly acidic conditions (pH 6.4–6.6). The inactivity of acetamide **6** which lacks features required for H-bonding, however, cannot readily be explained at present. Biphenyl **11** which shows good activity in the biological assays has the two aromatic rings almost perpendicular to one another, adopting therefore a conformation which is more favorable for binding to tubulin.

In summary, the biphenyls that we have prepared and examined thus far demonstrate that a single MeO substituent in the second phenyl ring must be at the 4′-position for the compound to have significant biological activity. Additional small alkyl groups at C(2′) further enhance activity (**3** and **11**) but eliminate it when introduced at C(5) (**26**). The good binding affinity of the 2′-ethyl-substituted biphenyl **11**, matching in potency that of well known phenyltropolones [6] [12] [13] and theoretically further enhancable by optical resolution [5], suggests that the 2′-position in 2,3,4,4′-tetramethoxybiphenyls is amenable to considerable changes. Nonetheless, there are definite limits regarding acceptable substituents at the 2′-position as demonstrated by the inactivity of **5**, **6**, and **8** as well as of additional compounds described previously [4]. Definitive conclusions as to the nature of 2′-substituents which enhance activity requires additional agents, and the synthesis of such compounds is underway.

Experimental Part

General. TLC: silica gel *GHLF* plates from *Analtech;* visualization with UV light, phosphomolybdic acid, I_2, $FeCl_3$ soln.; R_f data for $CHCl_3$/MeOH 9:1. Flash chromatography (FC): silica gel *60 (Merck)*, 230–400 mesh, 60

Å. M.p.: *Fisher-Johns* melting-point apparatus. IR spectra: *Beckman IR 4230* (cm^{-1}). ^{1}H-NMR spectra: *Varian XL 300* (300 MHz). MS: *Finingan 1015 D* instrument (CI).

*2',3',4,4'-Tetramethoxy-*N*-methyl-1,1'-biphenyl-2-methylamine* (**5**). To a soln. of $MeNH_2 \cdot HCl$ (201 mg) in MeOH (15 ml), 2',3',4,4'-tetramethoxy-1,1'-biphenyl-2-carbaldehyde (**4**; 150 mg) and $NaBH_3CN$ (19 mg) were added in one portion at r.t. and stirred overnight. Evaporation of the MeOH gave a residue, which was made alkaline with 5% NaOH soln. and extracted with $CHCl_3$. The extract was dried ($MgSO_4$) and evaporated. The oily residue was purified by FC ($CHCl_3$/MeOH 30:1): **5** (91 mg, 57.8%). The HCl salt of **5** was recrystallized from Et_2O. **5**·HCl: M.p. 167°. IR ($CHCl_3$): 2960, 2440, 1615, 1600. ^{1}H-NMR ($CDCl_3$): 7.63 (*d*, *J* = 2.4, H–C(3)); 7.16 (*d*, *J* = 8.4, H–C(6)); 6.96 (*dd*, *J* = 2.4, 8.4, H–C(5)); 6.86 (*d*, *J* = 8.5, H–C(6')); 6.76 (*d*, *J* = 8.5, H–C(5')); 4.02–3.96 (*m*, CH_2–C(2)); 3.93 (*s*, MeO); 3.91 (*s*, MeO); 3.89 (*s*, MeO); 3.58 (*s*, MeO); 2.53 (*s*, MeN). MS: 318 ($M^+ + 1$).

*2',3',4,4'-Tetramethoxy-*N*-acetyl-1,1'-biphenyl-2-methylamine* (**6**). A mixture of **5** (30 mg) and Ac_2O (0.3 ml) in pyridine (0.3 ml) was allowed to stand at r.t. for 16 h, then poured into H_2O, and extracted with Et_2O. The Et_2O extract was washed with 3% HCl and 5% $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated: **6** (32.5 mg, 95.7%) as an oil. IR ($CHCl_3$): 1637, 1600. ^{1}H-NMR: mixture of the (*E*)- and (*Z*)-isomer. MS: 360 ($M^+ + 1$).

N*-Benzyl-2',3',4,4'-tetramethoxy-1,1'-biphenyl-2-methylamine* (**7**). To a soln. of benzylamine hydrochloride (670 mg) in MeOH (30 ml), **4** (234 mg) and $NaBH_3CN$ (30 mg) were added in one portion at r.t. and stirred for 17 h. Evaporation of MeOH gave a residue which was made alkaline with 5% NaOH soln. and extracted with $CHCl_3$. The extract was dried ($MgSO_4$) and evaporated. The oily residue was purified by FC ($CHCl_3$/MeOH 99:1): **7** (256 mg, 84.1%) as an oil. IR ($CHCl_3$): 1600. ^{1}H-NMR ($CDCl_3$): 7.24–7.19 (*m*, $C_6H_5CH_2$); 7.12 (*d*, *J* = 8.3, H–C(6)); 7.09 (*d*, *J* = 2.7, H–C(3)); 6.85 (*d*, *J* = 8.5, H–C(6')); 6.83 (*dd*, *J* = 2.7, 8.3, H–C(5)); 6.69 (*d*, *J* = 8.5, H–C(5')); 3.90 (*s*, MeO); 3.88 (*s*, MeO); 3.86 (*s*, MeO); 3.67 (*s*, CH_2); 3.62 (*s*, CH_2); 3.54 (*s*, MeO). MS: 394 ($M^+ + 1$).

*2',3',4,4'-Tetramethoxy-*N*-benzyl-1,1'-biphenyl-2-methylamine* (**8**). A mixture of **7** (550 mg), 37% HCHO soln. (3 ml), and HCOOH (3 ml) was stirred at 90° for 4 h. After cooling, the mixture was made alkaline with 5% NaOH soln. and extracted with $CHCl_3$. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was chromatographed ($CHCl_3$): oily **8** (540 mg, 94.8%). IR ($CHCl_3$): 1600. ^{1}H-NMR ($CDCl_3$): 7.33 (*d*, *J* = 2.7, H–C(3)); 7.28–7.22 (*m*, $C_6H_5CH_2$); 7.11 (*d*, *J* = 8.4, H–C(6)); 6.83 (*d*, *J* = 8.6, H–C(6')); 6.81 (*dd*, *J* = 2.7, 8.4, H–C(5)); 6.70 (*d*, *J* = 8.6, H–C(5')); 3.92 (*s*, MeO); 3.91 (*s*, MeO); 3.87 (*s*, MeO); 3.52 (*s*, MeO); 3.39 (*s*, 2 CH_2); 2.06 (*s*, MeN). MS: 408 ($M^+ + 1$).

2',3',4,4'-Tetramethoxy-α-methyl-1,1'-biphenyl-2-methanol (**9**). To a stirred soln. of **4** (60 mg) in Et_2O (20 ml), 1.6M MeLi/Et_2O (0.2 ml) was added dropwise at r.t. under N_2 and stirred for 30 min. The mixture was washed with H_2O, dried ($MgSO_4$), and evaporated: oily **9** (61 mg, 96.6%). IR ($CHCl_3$): 3530, 3480, 1600. ^{1}H-NMR ($CDCl_3$): 7.18 (*d*, *J* = 2.6, H–C(3)); 7.10 (*d*, *J* = 8.4, H–C(6)); 6.87 (*dd*, *J* = 2.6, 8.4, H–C(5)); 6.87 (*d*, *J* = 8.6, H–C(6')); 6.75 (*d*, *J* = 8.6, H–C(5')); 4.67 (*q*, *J* = 6.1, CH–C(2)); 3.94 (*s*, MeO); 3.91 (*s*, MeO); 3.88 (*s*, MeO); 3.52 (*s*, MeO); 1.36 (*d*, *J* = 6.1, Me–C). MS: 301 ($M^+ - OH$).

2,3,4,4'-Tetramethoxy-2'-vinyl-1,1'-biphenyl (**10**). A soln. of **9** (54 mg) and conc. H_2SO_4 (4 drops) in 98% EtOH (5 ml) was refluxed for 12 h under N_2. After cooling, the mixture was poured into 5% $NaHCO_3$ soln. and extracted with Et_2O. The extract was washed with 5% $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated: **10** (48 mg). Crude **10** was used in the next step without further purification. An anal. sample was purified by FC ($CHCl_3$). IR ($CHCl_3$): 1605. MS: 301 ($M^+ + 1$).

2'-Ethyl-2,3,4,4'-tetramethoxy-1,1'-biphenyl (**11**). A mixture of **10** (48 mg) and Pd black (100 mg) in MeOH (10 ml) was hydrogenated at r.t./1 atm for 40 h. The catalyst was filtered and washed with MeOH. The combined filtrate and washings were evaporated. The residue was purified by FC ($CHCl_3$): **11** (42 mg, 81.9% from **9**). M.p. 75°. IR ($CHCl_3$): 1605. ^{1}H-NMR ($CDCl_3$): 7.09 (*d*, *J* = 8.3, H–C(6')); 6.85 (*d*, *J* = 2.7, H–C(3')); 6.83 (*d*, *J* = 8.5, H–C(6)); 6.76 (*dd*, *J* = 2.7, 8.3, H–C(5')); 6.70 (*d*, *J* = 8.5, H–C(5)); 3.92 (*s*, MeO); 3.90 (*s*, MeO); 3.85 (*s*, MeO); 3.58 (*s*, MeO); 2.48 (*q*, *J* = 7.5, CH_3CH_2); 1.08 (*t*, *J* = 7.5, $CHCH_2$). MS: 303 ($M^+ + 1$).

*2'-[(*N*-Benzyl-*N*-methylamino)methyl]-4,4',5,6-tetramethoxy-1,1'-biphenyl-3-methanol* (**12**). A soln. of **8** (400 mg) and 3.5% HCl soln. (2 ml) in 37% HCHO soln. (10 ml) was stirred at 130° for 45 h. After cooling, the mixture was made alkaline with 5% NaOH soln. and extracted with $CHCl_3$. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was chromatographed ($CHCl_3$): oily **12** (338 mg, 78.7%). IR ($CHCl_3$): 3600, 1608. ^{1}H-NMR ($CDCl_3$): 7.32 (*d*, *J* = 2.7, H–C(3')); 7.25–7.22 (*m*, $C_6H_5CH_2$); 7.10 (*d*, *J* = 8.5, H–C(6')); 6.83 (*s*, H–C(2)); 6.82 (*dd*, *J* = 2.7, 8.5, H–C(5')); 4.65 (*s*, CH_2–C(3)); 4.01 (*s*, MeO); 3.94 (*s*, MeO); 3.87 (*s*, MeO); 3.49 (*s*, MeO); 3.39 (*s*, 2 CH_2); 2.05 (*s*, MeN). MS: 438 ($M^+ + 1$).

N*-Benzyl-5'-(chloromethyl)-2',3',4,4'-tetramethoxy-*N*-methyl-1,1'-biphenyl-2-methylamine* (**13**). A mixture of **12** (318 mg) and $SOCl_2$ (0.1 ml) in benzene (5 ml) was refluxed for 2 h under N_2. After cooling, benzene and excess $SOCl_2$ were evaporated. The residue was made alkaline with 5% NaOH soln. and extracted with $CHCl_3$. The

extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was purified by chromatography ($CHCl_3$): **13** (237 mg, 71.5%) as an oil. IR ($CHCl_3$): 1605. ^{1}H-NMR ($CDCl_3$): 7.30–7.22 (*m*, $C_6H_5CH_2$, H–C(3)); 7.11 (*d*, J = 8.5, H–C(6)); 6.91 (*s*, H–C(6′)); 6.82 (*dd*, J = 2.8, 8.5, H–C(5)); 4.61 (*s*, CH_2–C(5′)); 4.03 (*s*, MeO); 3.93 (*s*, MeO); 3.87 (*s*, MeO); 3.51 (*s*, MeO); 3.39 (*s*, 2 CH_2); 2.05 (*s*, MeN). MS: 456 (M^+ + 1).

*2′,3′,4,4′-Tetramethoxy-*N,*5′-dimethyl-1,1′-biphenyl-2-methylamine* (**14**). A mixture of **13** (70 mg) and Pd black (40 mg) in AcOH (1 ml) was hydrogenated at r.t./1 atm for 3 h. The catalyst was filtered and the filtrate made alkaline with 5% NaOH soln. and extracted with $CHCl_3$. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated: **14**. The HCl salt of **14** (49 mg, 86.8%) was recrystallized from Et_2O. **14**·HCl: M.p. 122–123°. IR ($CHCl_3$): 2960–2230, 1610. ^{1}H-NMR ($CDCl_3$): 7.62 (*d*, J = 2.4, H–C(3)); 7.17 (*d*, J = 8.4, H–C(6)); 6.97 (*dd*, J = 2.4, 8.4, H–C(5)); 6.70 (*s*, H–C(6′)); 4.08–3.86 (*m*, CH_2–C(2)); 3.96 (*s*, MeO); 3.92 (*s*, MeO); 3.90 (*s*, MeO); 3.54 (*s*, MeO); 2.59 (*s*, MeN); 2.24 (*s*, Me–C(5′)). MS: 332 (M^+ + 1).

N-*Acetyl-2′,3′,4,4′-tetramethoxy-*N,*5′-dimethyl-1,1′-biphenyl-2-methylamine* (**15**). A soln. of **14** (16 mg) and Ac_2O (0.5 ml) in pyridine (0.5 ml) was kept at r.t. for 16 h. The mixture was poured into H_2O and extracted with Et_2O. The extract was washed with 3% HCl and 5% $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated: **15** (14 mg, 86.2%). IR ($CHCl_3$): 1630, 1610. ^{1}H-NMR: mixture of the (*E*)- and (*Z*)-isomer. MS: 374 (M^+ + 1).

Methyl 5′-(Hydroxymethyl)-2′,3′,4,4′-tetramethoxy-1,1′-biphenyl-2-carboxylate (**17**). A soln. of methyl 2′,3′,4,4′-tetramethoxy-1,1′-biphenyl-2-carboxylate (**16**; 150 mg), 37% HCHO (1.5 ml) and 3.5% HCl soln. (0.5 ml) in diglyme (1.5 ml) was stirred at 120° for 20 h under N_2. After cooling, the mixture was poured into H_2O and extracted with $CHCl_3$. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was chromatographed ($CHCl_3$): oily **17** (117 mg, 71.5%) as an oil. IR ($CHCl_3$): 1720, 1605. ^{1}H-NMR ($CDCl_3$): 7.42 (*d*, J = 2.8, H–C(3)); 7.25 (*d*, J = 8.5, H–C(6)); 7.07 (*dd*, J = 2.8, 8.5, H–C(5)); 6.93 (*s*, H–C(6′)); 4.67 (*d*, J = 6.1, CH_2–C(5′)); 3.99 (*s*, MeO); 3.92 (*s*, MeO); 3.88 (*s*, MeO); 3.68 (*s*, MeO); 3.51 (*s*, MeO). MS: 345 (M^+ – OH).

Methyl 2′,3′,4,4′-Tetramethoxy-5′-methyl-1,1′-biphenyl-2-carboxylate (**19**). A soln. of **17** (112 mg) and $SOCl_2$ (150 µl) in benzene (3 ml) was refluxed for 3 h under N_2. After cooling, benzene and excess $SOCl_2$ were evaporated to given an oily residue which was partitioned between H_2O and Et_2O. The org. layer was washed with H_2O, dried ($MgSO_4$), and evaporated: crude *methyl 5′-(chloromethyl)-2′,3′,4,4′-tetramethoxy-1,1′-biphenyl-2-carboxylate* (**18**, 115 mg). A mixture of crude **18** (115 mg) and Pd black (40 mg) in MeOH (5 ml) was hydrogenated at r.t./1 atm for 1.5 h. The catalyst was filtered and washed with MeOH. The combined filtrate and washings were evaporated. The residue was chromatographed ($CHCl_3$): **19** (26 mg, 24.3%) as an oil. IR ($CHCl_3$): 1725, 1607. ^{1}H-NMR ($CDCl_3$): 7.40 (*d*, J = 2.7, H–C(3)); 7.25 (*d*, J = 8.5, H–C(6)); 7.07 (*dd*, J = 2.7, 8.5, H–C(5)); 6.77 (*s*, H–C(6′)); 3.92 (*s*, MeO); 3.89 (*s*, MeO); 3.88 (*s*, MeO); 3.67 (*s*, MeO); 3.48 (*s*, MeO); 2.24 (*s*, Me–C(5′)). MS: 347 (M^+ + 1).

Methyl 5′-Formyl-2′,3′,4,4′-tetramethoxy-1,1′-biphenyl-2-carboxylate (**20**) *and 4,4′,5,6-Tetramethoxy-2′-(methoxycarbonyl)-1,1′-biphenyl-3-carboxylic Acid* (**21**). To a stirred soln. of **17** (85 mg) in acetone (0.5 ml), *Jones* reagent (80 mg) was added dropwise at 0° and stirred at r.t. for 15 min. Then, the mixture was poured into H_2O and extracted with Et_2O. The extract was washed with H_2O and 5% $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated. The residue was purified by FC ($CHCl_3$): oily **20** (45 mg, 51.0%). IR ($CHCl_3$): 1720, 1680, 1605, 1590. ^{1}H-NMR ($CDCl_3$): 10.32 (*s*, CHO); 7.49 (*s*, H–C(6′)); 7.46 (*d*, J = 2.7, H–C(3)); 7.22 (*d*, J = 8.4, H–C(6)); 7.09 (*dd*, J = 2.7, 8.4, H–C(5)); 4.08 (*s*, MeO); 3.92 (*s*, MeO); 3.89 (*s*, MeO); 3.70 (*s*, MeO); 3.66 (*s*, MeO). MS: 361 (M^+ + 1).

The aq. 5% $NaHCO_3$ layer was acidified with 3% HCl soln. and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried ($MgSO_4$), and evaporated: **21** (26 mg, 29.4%). M.p. 129°. IR ($CHCl_3$): 3230–2220, 1725, 1600. ^{1}H-NMR ($CDCl_3$): 11.15 (br. *s*, COOH); 7.80 (*s*, H–C(2)); 7.47 (*d*, J = 2.8, H–C(3′)); 7.23 (*d*, J = 8.6, H–C(6′)); 7.10 (*dd*, J = 2.8, 8.6, H–C(5′)); 4.19 (*s*, MeO); 3.92 (*s*, MeO); 3.89 (*s*, MeO); 3.71 (*s*, MeO); 3.64 (*s*, MeO). MS: 377 (M^+ + 1).

2′,3′,4,4′-Tetramethoxy-5′-methyl-1,1′-biphenyl-2-methanol (**22**). To a stirred suspension of $LiAlH_4$ (5 mg) in Et_2O (1 ml), a soln. of **19** (25 mg) in Et_2O (2 ml) was added dropwise at 0° and stirred at r.t. for 30 min under N_2. To this mixture, Et_2O which was saturated with H_2O was added dropwise. Then, the mixture was washed with H_2O, dried ($MgSO_4$), and evaporated: **22** (22 mg, 95.7%) as an oil. IR ($CHCl_3$): 3540, 3490, 1610. ^{1}H-NMR ($CDCl_3$): 7.08 (*d*, J = 8.5, H–C(6)); 7.01 (*d*, J = 2.7, H–C(3)); 6.83 (*dd*, J = 2.7, 8.5, H–C(4)); 6.64 (*s*, H–C(6′)); 4.28 (*s*, CH_2–C(2)); 3.89 (*s*, MeO); 3.83 (*s*, MeO); 3.80 (*s*, MeO); 3.42 (*s*, MeO); 2.17 (*s*, Me–C(5′)). MS: 318 (M^+).

2′,3′,4,4′-Tetramethoxy-5′-methyl-1,1′-biphenyl-2-carbaldehyde (**23**). To a stirred suspension of pyridinium chlorochromate (24 mg) in CH_2Cl_2 (0.5 ml), a soln. of **22** (21 mg) in CH_2Cl_2 (1 ml) was added in one portion at r.t. and stirred for 1.5 h. Et_2O (2 ml) was added and the supernatant liquid decanted from a black gum. The insoluble residue was washed with Et_2O. The combined org. soln. was passed through a short pad of *Florisil* and evaporated: **23** (17 mg, 81.5%) as an oil. IR ($CHCl_3$): 1685, 1605. ^{1}H-NMR ($CDCl_3$): 9.80 (*s*, CHO); 7.49 (*d*, J = 2.8, H–C(3)); 7.29 (*d*, J = 8.4, H–C(6)); 7.18 (*dd*, J = 2.8, 8.4, H–C(5)); 6.80 (*s*, H–C(6′)); 3.93 (*s*, MeO); 3.91 (*s*, MeO); 3.89 (*s*, MeO); 3.47 (*s*, MeO); 2.25 (*s*, Me–C(5′)). MS: 317 (M^+ + 1).

Compound **14** *from* **23**. To a stirred soln. of $MeNH_2 \cdot HCl$ (18 mg) in MeOH (1 ml), **23** (14 mg) and $NaBH_3CN$ (2 mg) were added in one portion at r.t. and stirred for 20 h. Evaporation of MeOH gave a residue which was made alkaline with 5% NaOH soln. and extracted with $CHCl_3$. The extract was dried ($MgSO_4$) and evaporated. The residue was chromatographed ($CHCl_3$/MeOH 98:2): **14** (9 mg, 61.4%). All spectral data of **14** were identical with those of **14** obtained from **4** *via* **8** and **12**.

4,4',5',6'-Tetramethoxy-1,1'-biphenyl-2,3'-dimethanol (**24**). To a stirred suspension of $LiAlH_4$ (5 mg) in Et_2O (5 ml), a soln. of **17** (50 mg) in Et_2O (5 ml) was added dropwise at 0° and stirred at r.t. for 30 min under N_2. Then, H_2O-sat. Et_2O was added dropwise and the mixture washed with H_2O and dried ($MgSO_4$). Evaporation gave oily **24** (44 mg, 95.4%). IR ($CHCl_3$): 3610, 3470, 1605. ^{1}H-NMR ($CDCl_3$): 7.15 (*d*, J = 8.5, H–C(6)); 7.10 (*d*, J = 2.7, H–C(3)); 6.95 (*dd*, J = 2.7, 8.5, H–C(5)); 6.88 (*s*, H–C(2')); 4.66 (*d*, J = 6.1, CH_2–C); 4.36 (*s*, CH_2–C); 4.01 (*s*, MeO); 3.96 (*s*, MeO); 3.87 (*s*, MeO); 3.52 (*s*, MeO). MS: 317 (M^+ – OH).

4,4',5',6'-Tetramethoxy-1,1'-biphenyl-2,3'-dicarbaldehyde (**25**). To a stirred suspension of pyridium chlorochromate (90 mg) in CH_2Cl_2 (1 ml) a soln. of **24** (40 mg) in CH_2Cl_2 (1 ml) was added in one portion at r.t. and stirred for 1.5 h. Et_2O (2 ml) was added and the supernatant liquid decanted from a black gum. The insoluble residue was washed with Et_2O. The combined org. soln. was passed through a short pad of *Florisil* and evaporated: oily **25** (36 mg, 91.1%). IR ($CHCl_3$): 1690, 1605, 1595. ^{1}H-NMR ($CDCl_3$): 10.35 (*s*, CHO); 9.77 (*s*, CHO); 7.56 (*s*, H–C(2')); 7.50 (*d*, J = 2.7, H–C(3)); 7.27 (*d*, J = 8.5, H–C(6)); 7.24 (*dd*, J = 2.7, 8.5, H–C(5)); 4.11 (*s*, MeO); 3.95 (*s*, MeO); 3.91 (*s*, MeO); 3.67 (*s*, MeO). MS: 331 (M^+ + 1).

2,3,4,4'-Tetramethoxy-2',5-dimethyl-1,1'-biphenyl (**26**). A mixture of **25** (60 mg), 80% hydrazine hydrate (0.05 ml), and KOH (50 mg) in diethylene glycol (1 ml) was stirred for 40 min at 150°. After cooling, the mixture was poured into H_2O and extracted with Et_2O. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The oily residue was chromatographed ($CHCl_3$): **26** (25 mg, 45.5%) as a crystal. M.p. 69°. IR ($CHCl_3$): 1605. ^{1}H-NMR ($CDCl_3$): 7.10 (*d*, J = 8.3, H–C(6')); 6.80 (*d*, J = 2.7, H–C(3')); 6.77 (*dd*, J = 2.7, 8.3, H–C(5')); 6.67 (*s*, H–C(6)); 3.94 (*s*, MeO); 3.90 (*s*, MeO); 3.83 (*s*, MeO); 3.51 (*s*, MeO); 2.23 (*s*, Me–C); 2.6 (*s*, Me–C). MS: 303 (M^+ + 1).

4,4',5',6'-Tetramethoxy-1,1'-biphenyl-2,3'-dicarboxylic Acid (**27**). A soln. of **21** (15 mg) and 5% NaOH soln. (0.5 ml) in MeOH (2 ml) was stirred at 70° for 8 h. After cooling, the MeOH was evaporated and the residue acidified with 3% HCl soln. and extracted with $CHCl_3$. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated: **27** (12 mg, 83.1%). M.p. 189°. IR ($CHCl_3$): 3400–2300, 1740, 1695, 1610. ^{1}H-NMR ($CDCl_3$): 7.78 (*s*, H–C(2')); 7.53 (*d*, J = 2.7, H–C(3)); 7.24 (*d*, J = 8.5, H–C(6)); 7.14 (*dd*, J = 2.7, 8.5, H–C(5)); 4.17 (*s*, MeO); 3.89 (*s*, MeO); 3.86 (*s*, MeO); 3.66 (*s*, MeO). MS: 363 (M^+ + 1).

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