A Practical Synthesis of the Anti-tumour Agent 2-Methyl-4-deoxyisopicropodophyllotoxin and Related Podophyllin Analogues

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We describe an efficient, six-stage synthesis of 2-methyl-4-deoxyisopicropodophyllotoxin, which proceeds *via* a Diels–Alder cycloaddition between 2-methylmaleic anhydride and the orthoquinodimethane generated by treatment of 5-[1-acetoxy-1-(3',4',5'-trimethoxyphenyl)methyl]-6-trimethylsilylmethyl-1,3-benzodioxole with silica gel at 60 °C. The podophyllin was converted into a number of 4'-ester derivatives. This procedure was also used to prepare a diazapodophyllin *via* the use of 4-phenyl-1,2,4-triazoline-3,5-dione as dienophile.

Extracts of the plants *Podophyllum peltatum* and *P. emodii* have long been valued for the treatment of warts and other benign tumours, and their major constituents, the aryltetralin lignans podophyllotoxin **1** and beta-peltatin **2**, have proven activity as inhibitors of cell division.[1] Two semi-synthetic derivatives of **1**, etoposide **3** and teniposide **4**, are now widely and successfully used for the treatment of, *inter alia*, small cell lung cancer, testicular teratoma and refractory childhood leukaemia.[2]

In 1985 we described a route to 2-substituted-4-deoxy-analogues of podophyllotoxin **5** which proceeded *via* cycloaddition reactions between the orthoquinodimethane **6** and dienophiles **7** (Scheme 1).[3] We went on to show that several of these analogues, most notably **5a**, exhibited marked *in vivo* antitumour activity[4] despite possessing a *cis*-lactone ring rather than the *trans*-ring of podophyllotoxin. Computer modelling of the various X-ray structures for analogues **5** demonstrated that they all possessed very similar three-dimensional structures to podophyllotoxin. In particular, the pendant aryl rings were perpendicular to the rather flattened ABCD ring systems, a structural feature that appears to be important for anti-mitotoic activity.[5]

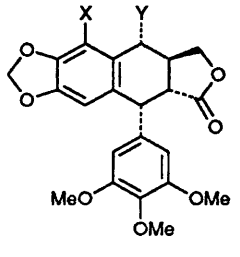
The route shown in Scheme 1 could not, however, be used for the large-scale synthesis of podophyllins. In order to overcome this problem, we devised a milder and more practical route to aryltetralin lignans of this type, and the overall route is shown in Scheme 2. During the course of this work, Takano and co-workers reported a conceptually similar route (shown in Scheme 3),[6] but a full account of this work has not yet appeared, so the practical utility of the methodology cannot be assessed. In addition, our method employs silica as the 'trigger' for the cycloaddition, whereas the Japanese workers used the dienophile maleic anhydride as 'trigger'.

The requisite orthoquinodimethane precursor **12** was prepared in four steps from piperonyl bromide **8**. Reaction of this with magnesium turnings (previously activated by vigorous overnight stirring under nitrogen)[7] in the presence of trimethylsilyl chloride, provided the silane **9**, which was immediately brominated to yield the bromo silane **10** (27% overall yield for the two steps on the 0.4 molar scale). Metal–halogen exchange was effected with butyllithium and the resultant aryllithium was treated with 3,4,5-trimethoxybenzaldehyde to yield the alcohol **11** (50–70% on the 0.1 molar scale). This was then acetylated to provide the desired orthoquinodimethane precursor **12**.

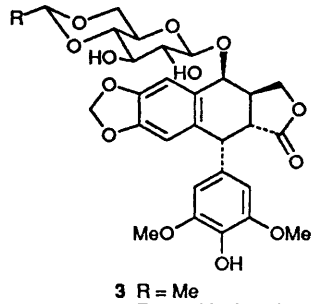
We envisaged that this would react with fluoride ion to produce the orthoquinodimethane **6**, but attempts to achieve this transformation with tetrabutylammonium fluoride and caesium fluoride in the presence of the dienophile 2-methylmaleic anhydride (citraconic anhydride) produced intractable mixtures of products. However, during attempted purification of the acetate **12** on silica gel, we noticed a blue coloration on the column, and subsequently were able to isolate an inseparable mixture of the two compounds **13** and **14** in the eluent. These are products of intramolecular cycloaddition of the required orthoquinodimethane **6**. Presumably the silica acts as both Lewis acid and source of nucleophiles. This serendipitous observation coupled with some process development led to the successful methodology shown in Scheme 2. The reaction with citraconic anhydride has been carried out routinely and reproducibly on the 30 mmol scale using this methodology to yield the cycloadduct **15** in yields of 70–75% after chromatography. Much smaller amounts (never greater than 10%) of the alternative regioisomer **16** were also isolated.

There has been considerable interest recently in the preparation of aza- and diaza-podophyllins, *e.g.* **17**[8] and **18**,[9] and it was thus of interest to attempt cycloadditions with diaza-dienophiles. Under our standard reaction conditions we could not obtain any cycloadducts with diethyl azodicarboxylate, but use of 4-phenyl-1,2,4-triazoline-3,5-dione **19** provided the cycloadduct **20** in 28% isolated yield.

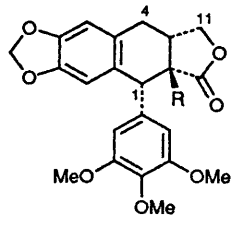
Finally, in order to extend our knowledge of the structure–biological activity profiles of these podophyllin analogues, we converted the cycloadduct **15** into lactone **5a** using K-selectride in THF followed by treatment with acid.[4] This lactone could be



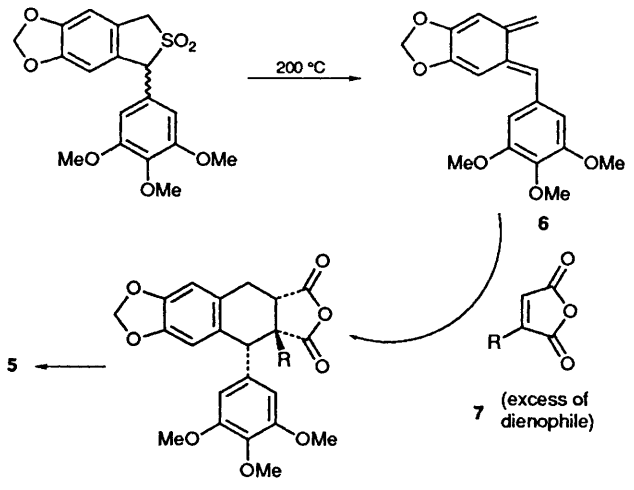
1 X = H, Y = OH
2 X = OH, Y = H



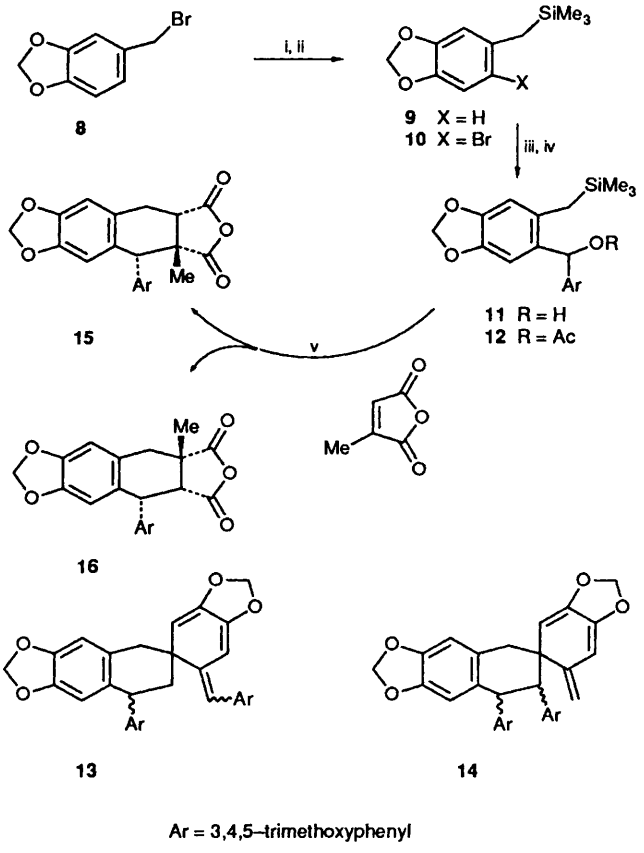
3 R = Me
4 R = 2-thiophenyl



5a R = Me, **b** R = CF_3, **c** R = Ph



Scheme 1



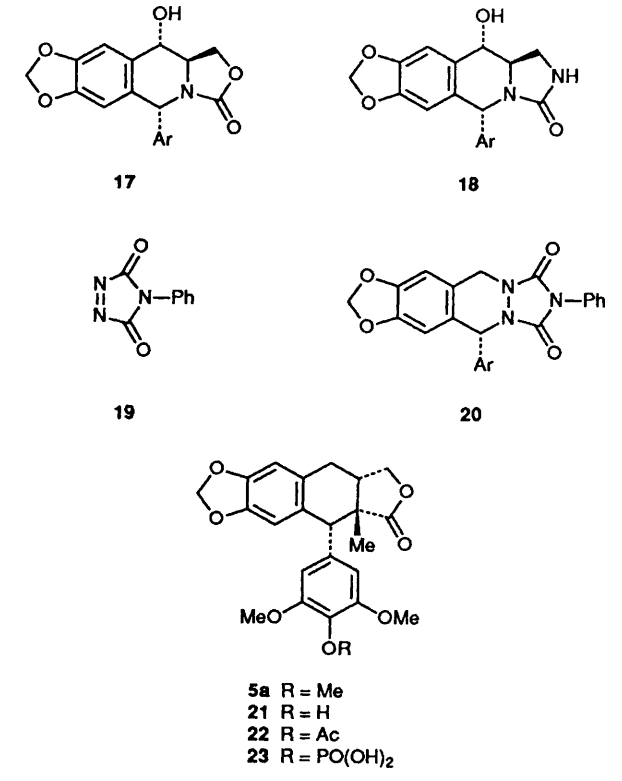
Scheme 2 *Reagents and conditions:* i, Mg–TMSCl–THF; ii, Br_2–10% $NaHCO_3$–dichloromethane; iii, BuLi–THF then 3,4,5-trimethoxybenzaldehyde; iv, Ac_2O–pyridine; v, SiO_2–CCl_4, 50° C

selectively demethylated using the method of Kuhn[10] (HBr in dichloroethane) to produce 2-methyl-4-deoxy-4'-demethylisopicropodophyllotoxin **21**. This was subsequently converted into the acetate **22** and phosphate **23**. These analogues were of considerable interest given the potent anti-cancer activity of etoposide **3** and teniposide **4**, both of which lack the 4'-methyl group of podophyllotoxin **1**.

The four new compounds **20–23** were screened for cytotoxic activity* against cultures of human colorectal tumour cells (LS174T) and human choriocarcinoma tumour cells (JAR). None of the compounds exhibited any activity against the JAR cells, but compound **21** had an IC_{50} value (it caused 50% inhibition of growth) at 0.082 μmol dm^{-3} concentration against



Scheme 3 *Reagent and conditions:* 5 equiv. maleic anhydride in refluxing toluene 24 h (64%)



the LS174T cells. Etoposide had an IC_{50} of 0.81 μmol dm^{-3} against the same cell line. This interesting result will need confirmation, and further biological evaluation is underway.

In a recent expert review of synthetic approaches to podophyllotoxin and analogues, R. S. Ward concluded that 'no totally satisfactory (synthetic) solution has so far been found and that ingenuity has not been exhausted'.[11] The approach reported in this paper will, we believe, provide ready access to a range of novel, therapeutically useful podophyllin analogues. It should also lend itself to the production of stereochemically pure compounds through the use of chiral dienophiles, and this possibility is under active investigation.

Experimental

IR spectra were recorded on a Perkin-Elmer 881 double beam grating spectrometer using sodium chloride cells and chloroform solutions unless otherwise stated. NMR spectra were recorded at 220 MHz using a Perkin-Elmer R34 instrument, and at 400 MHz *via* the SERC NMR Service at the University of Warwick using a Bruker WH 400 instrument. *J*-Values are given in Hz. M.p.s were determined using an Electrothermal digital apparatus and are uncorrected.

* The compounds were administered in DMSO to cultures containing 10^5 tumour cells, and incubation was continued for 5 days (JAR cells) or 7 days (LS174T cells). After addition of Trypan Blue, viable cells (those excluding the blue dye) were counted using a haemocytometer. We are enormously grateful to Professor E. S. Newlands and Dr. J. Baer, Charing Cross Hospital, for these preliminary results.

Reactions requiring anhydrous conditions were carried out under a static nitrogen atmosphere using oven-dried glassware. THF (tetrahydrofuran) was dried over molecular sieves and distilled from sodium–benzophenone prior to use. All other reagents and solvents were used as supplied without further purification. Flash chromatography was performed using Sorbosil C60 silica gel. Ether refers to diethyl ether and light petroleum refers to the fraction with b.p. 40–60 °C unless otherwise stated.

Piperonyl Bromide **8**.—Piperonyl alcohol (101.44 g, 0.067 mol) was added portionwise to HBr (49% aq., 100 cm^3), and the resultant suspension was allowed to stand at room temp. for 2 h. Dichloromethane (1 dm^3) was then added, and the organic layer separated. The aqueous layer was extracted with dichloromethane (3 × 800 cm^3), and the combined organic extract was washed with water (2 × 500 cm^3) and saturated aqueous sodium hydrogen carbonate (3 × 500 cm^3), dried and concentrated to yield the crude bromide (123.63 g, *ca.* 85%), which was used without further purification. An analytical sample was prepared by recrystallisation from light petroleum, m.p. 46–48 °C (lit.,[3] m.p. 48 °C); $\delta(CDCl_3$; 60 MHz) 4.43 (s, 2 H, CH_2Br), 5.9 (s, 2 H, OCH_2O) and 6.8 (m, 3 H, ArH).

5-*Bromo*-6-*trimethylsilylmethyl*-1,3-*benzodioxole* **10**.—Magnesium turnings (9.04 g, 0.38 mol) were activated by stirring overnight under a stream of nitrogen. Freshly distilled THF (75 cm^3) and freshly distilled trimethylsilyl chloride (48 cm^3, 0.038 mol) were added, followed by the dropwise addition of a solution of piperonyl bromide (73.62 g, 0.38 mol) in THF (175 cm^3) over a period of 1 h at 0 °C. After the addition was complete, the reaction mixture was poured into an ice–water mixture (1 dm^3), and the products were extracted with light petroleum (2 × 500 cm^3). The combined organic extract was dried and concentrated to yield the crude silane **9** (44.93 g). This was dissolved in dichloromethane (200 cm^3), and to this was added aqueous sodium carbonate (10%, 200 cm^3), followed by bromine (14 cm^3, 0.27 mol) in dichloromethane (50 cm^3), and the mixture held at 0 °C for 1 h. The organic layer was separated off, and the aqueous layer extracted with dichloromethane (2 × 200 cm^3). The combined organic extract was dried, concentrated, and the products separated by flash chromatography, eluting with light petroleum–ether (9:1) to yield the bromo silane **10** (25.87 g, 27% overall for two steps). R_f 0.75 (light petroleum–ether, 9:1); $\delta(CDCl_3$; 60 MHz) 0.0 (s, 9 H, $SiMe_3$), 2.15 (s, 2 H, CH_2TMS), 5.82 (s, 2 H, OCH_2O), 6.5 (s, 1 H, 7-H) and 6.85 (s, 1 H, 4-H); *m/z* 288.

5-[1-*Acetoxy*-1-(3′,4′,5′-*trimethoxyphenyl*)*methyl*]-6-*trimethylsilylmethyl*-1,3-*benzodioxole* **12**.—Butyllithium (40 cm^3 of a 2.5 mol dm^{-3} solution in hexane; 0.1 mol) was added dropwise to a stirred solution of the bromo silane **10** (25.39 g, 0.092 mol) in dry THF (400 cm^3) over a 40 min period with stirring at −78 °C. The resultant solution was stirred at the same temperature for 2.5 h prior to the dropwise addition of 3,4,5-trimethoxybenzaldehyde (25.05 g, 0.13 mol) in dry THF (100 cm^3). The mixture was then warmed to room temp. and stirred overnight. After addition of water (100 cm^3), the THF was removed under reduced pressure, and the residue was diluted with water (200 cm^3), and the products extracted into dichloromethane (3 × 250 cm^3). The combined organic extract was washed with brine (200 cm^3), dried and concentrated to yield the crude alcohol **11** (*ca.* 18 g, around 50%). The yield was increased to 70% when the reaction was carried out on an 18 g scale. The alcohol (15.11 g, 37 mmol) was dissolved in pyridine (50 cm^3) and acetylated with acetic anhydride (3.62 cm^3, 44.5 mol) at 0 °C. The reaction was complete after 24 h at room temp., and the mixture was partitioned between water (150 cm^3) and light petroleum (150 cm^3). The organic layer was separated and the aqueous layer was extracted with light petroleum (2 × 150 cm^3). The combined organic extract was washed with 2 mol dm^{-3} HCl (2 × 150 cm^3), brine (150 cm^3), dried and concentrated to yield the acetate **12**. Recrystallisation from light petroleum provided pure product (9.85 g, 59%). R_f 0.4 (light petroleum–ether 2:1); m.p. 99–101 °C; ν_{max}/cm^{-1} 2955, 2865, 2839, 1728, 1598, 1502, 1482, 1369, 1235, 1019, 869 and 692; $\delta(CDCl_3$; 220 MHz) 0.0 (s, 9 H, Me_3Si), 2.05 (s, 2 H, CH_2TMS), 2.15 (s, 3 H, OAc), 3.8 (s, 6 H, 3′-OMe and 5′-OMe), 3.82 (s, 3 H, 4′-OMe), 5.95 (s, 2 H, OCH_2O), 6.5 (s, 1 H, CHO), 6.52 (s, 2 H, 2′-H and 6′-H), 6.85 (s, 1 H, 7-H) and 6.9 (s, 1 H, 4-H) (Found: C, 61.85; H, 6.75. $C_{23}H_{30}O_7Si$ requires C, 61.89; H, 7.01%).

3a,4-trans-3a,9a-cis-3a-*Methyl*-6,7-*methylenedioxy*-4-(3′,4′,5′-*trimethoxyphenyl*)-3a,4,9,9a-*tetrahydronaphtho*[2,3-c]*furan*-1,3-*dione* **15**.—A mixture of the acetate **12** (12.58 g, 28.2 mmol), citraconic anhydride (6.34 g, 56.57 mmol) and flash silica (10 g) was stirred in CCl_4 (200 cm^3) at 50 °C for 10 d. The resultant coloured solution was filtered through Celite, then partitioned between water (250 cm^3) and dichloromethane (250 cm^3). The organic layer was separated, and the aqueous layer extracted with dichloromethane (2 × 200 cm^3). The combined organic extract was washed with brine (200 cm^3), dried, and concentrated. The residue was purified by chromatography using ether–light petroleum (2:1) as eluent to yield the desired cycloadduct **15** (9.17 g, 76%); R_f 0.3 (light petroleum–ether, 2:1); m.p. 153 °C (lit.,[3] m.p. 153 °C); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2900, 1850, 1785, 1590, 1485, 1460, 1330, 1230, 1125, 1040, 1005, 960 and 865; $\delta(CDCl_3$; 400 MHz) 1.42 (s, 3 H, Me), 3.10 (dd, 1 H, *J* 15.9 and 9.8, 9_{alpha}-H), 3.18 (m, 1 H, 9a-H), 3.40 (dd, 1 H, *J* 15.9 and 3.2, 9_{beta}-H), 3.75 (s, 6 H, 3′-OMe and 5′-OMe), 3.80 (s, 3 H, 4′-OMe), 3.90 (s, 1 H, 4-H), 5.90 (s, 2 H, OCH_2O), 6.32 (s, 2 H, 2′-H and 6′-H), 6.58 (s, 1 H, 5-H) and 6.75 (s, 1 H, 8-H) (Found: C, 64.95; H, 5.25. $C_{23}H_{22}O_8$ requires C, 64.77; H, 5.20%).

2-*Methyl*-4-*deoxyisodeoxpicropodophyllotoxin* **5a**.—K-Selectride (22.52 cm^3 of a 1 mol dm^{-3} solution in THF, 22.5 mmol) was added dropwise to a stirred solution of the cycloadduct **15** (4.80 g, 11.3 mmol) in dry THF (80 cm^3) at −78 °C, and the resultant solution was stirred at −78 °C for 4.5 h. The reaction was quenched by the addition of water (12 cm^3), and after warming to room temp., 2 mol dm^{-3} NaOH (56 cm^3) was added followed by **CAREFUL** addition of hydrogen peroxide (34 cm^3, 30% solution) and the mixture was then stirred at room temp. overnight. 6 Mol dm^{-3} HCl (34 cm^3) was then added, and the solution was stirred for 15 min at room temp., and the products extracted into dichloromethane. This extract was dried, concentrated, and the residue taken up in THF (50 cm^3), prior to the addition of DCC (2.95 g). The solution was then heated under reflux for 3 h, prior to chromatography (ether–light petroleum, 2:1 as eluent) which provided the desired lactone **5a** (3.72 g, 80%); R_f 0.25 (ether–light petroleum, 2:1); m.p. 109–110 °C (lit.,[4] m.p. 108 °C); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2900, 1765, 1590, 1505, 1485, 1330, 1230, 1125 and 1040; $\delta(CDCl_3$; 400 MHz) 1.3 (s, 3 H, Me), 2.75 (m, 2 H, 3-H and 4_{alpha}-H), 3.0 (m, 1 H, 4_{beta}-H), 3.35 (dd, 1 H, *J* 10, 11_{beta}-H), 3.71 (s, 6 H, 3′-OMe and 5′-OMe), 3.77 (s, 3 H, 4′-OMe), 3.93 (s, 1 H, 1-H), 4.25 (m, 1 H, *J* 10, 11_{alpha}-H), 5.92 (s, 2 H, OCH_2O), 6.41 (s, 2 H, 2′-H and 6′-H), 6.59 (s, 1 H, 8-H) and 6.71 (s, 1 H, 5-H) (Found: C, 66.97; H, 5.82. $C_{23}H_{24}O_7$ requires C, 66.97; H, 5.87%).

2-*Methyl*-4-*deoxy*-4′-*demethylisopicropodophyllotoxin* **21**.—HBr was passed through a solution of the lactone **5a** (5.52 g, 13.4 mmol) in 1,2-dichloroethane–ether (10:1, 110 cm^3) at 0 °C. The resultant solution was allowed to stand at 4 °C for

3 d, prior to concentration and chromatography (ether–light petroleum, 3:1 as eluent) to yield the desired phenol **21** (2.84 g, 53%); R_f 0.25 (ether–light petroleum, 2:1); m.p. 170–174 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3536, 2965, 1759, 1616, 1516, 1481, 1424, 1325, 1218, 1116, 1021, 938, 861, 771 and 638; $\delta(CDCl_3$; 400 MHz) 1.32 (s, 3 H, Me), 2.72 (m, 2 H, 3-H and 4_{alpha}-H), 3.03 (m, 1 H, *J* 8.6, 4_{beta}-H), 3.28 (dd, *J* 8.8, 11_{beta}-H), 3.79 (s, 6 H, 3'-OMe and 5'-OMe), 3.98 (s, 1 H, 1-H), 4.25 (dd, 1 H, *J* 8.6, 11_{alpha}-H), 5.42 (br s, 1 H, OH), 5.76 (s, 2 H, OCH_2O), 6.42 (s, 2 H, 2'-H and 6'-H), 6.62 (s, 1 H, 8-H) and 6.72 (s, 1 H, 5-H) (Found: C, 65.5; H, 5.6. $C_{22}H_{22}O_7$ requires C, 65.58; H, 5.62%).

7,8-*Methylenedioxy*-2-*phenyl*-5-(3',4',5'-*trimethoxyphenyl*)-5,10-*dihydro*[1,2,4]*triazolo*[1,2-b]*phthalazine*-1,3(2H)-*dione* **20**.—4-Phenyl-1,2,4-triazoline-3,5-dione **19** (0.39 g, 2.2 mmol) was added to a stirred solution of the acetate **12** (0.5 g, 1.1 mmol) in carbon tetrachloride (10 cm^3) containing suspended silica. The resultant suspension was heated at 50 °C for 4 d. The reaction mixture was partitioned between water (50 cm^3), and dichloromethane (3 × 50 cm^3), and the combined organic extract was washed once with water (50 cm^3), dried and concentrated to yield an orange oil. This was purified by flash chromatography eluting with ether–light petroleum (2:1) to yield the cycloadduct **20** as a pale yellow foam (0.15 g, 28%); $\nu_{max}(CDCl_3)/cm^{-1}$ 2983, 1773, 1712, 1594, 1503, 1457, 1422, 1265, 1163, 1042, 936 and 851; $\delta(CDCl_3$; 220 MHz) 1.95 (d, 1 H, *J* 18, 10-H), 2.3 (d, 1 H, *J* 18, 10-H), 3.75 (s, 6 H, 3'- and 5'-OMe), 3.85 (s, 3 H, 4'-OMe), 5.9 (d, 2 H, *J* 2, OCH_2O), 6.4 (s, 2 H, 2'- and 6'-H), 6.49 (s, 1 H, 5-H), 6.5 (s, 1 H, 6-H), 6.65 (s, 1 H, 9-H) and 7.4–7.5 (m, 5 H, Ph) (Found: M^+, 489.1536. $C_{26}H_{23}N_3O_7$ requires *M*, 489.1535).

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