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A Convergent Synthesis of Mycophenolic Acid

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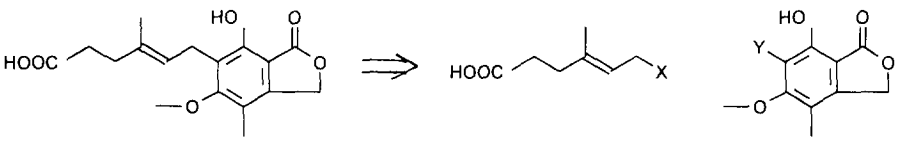
Abstract: A new method for the synthesis of Mycophenolic acid using a convergent approach has been developed where the key step is a palladium mediated allyl-aryl tin coupling.

Despite being a century old, Mycophenolic acid (MPA, 1), a mold metabolite from Penicillium brevi-compactum, is still the center of much interest and research.[1] Its remarkable affinity for inhibiting the enzyme inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the de-novo synthesis of GTP, makes it an attractive molecule for inhibiting cell growth in areas such as cancer and immunosupression. However, MPA has an extremely short biological half-life *in vivo* due in part to rapid glucuronidation of the phenol leading to biliary excretion.[1e] During the course of our research aimed at improving its pharmacokinetics, we required an efficient method for the synthesis of ring modified MPA analogs. A number of MPA total syntheses have been published since 1969[2] but none offered the convergent approach desired. The synthesis of MPA via a palladium-tin coupling reaction between the alkyl side chain 2 and the phthalide ring 3 is reported here.

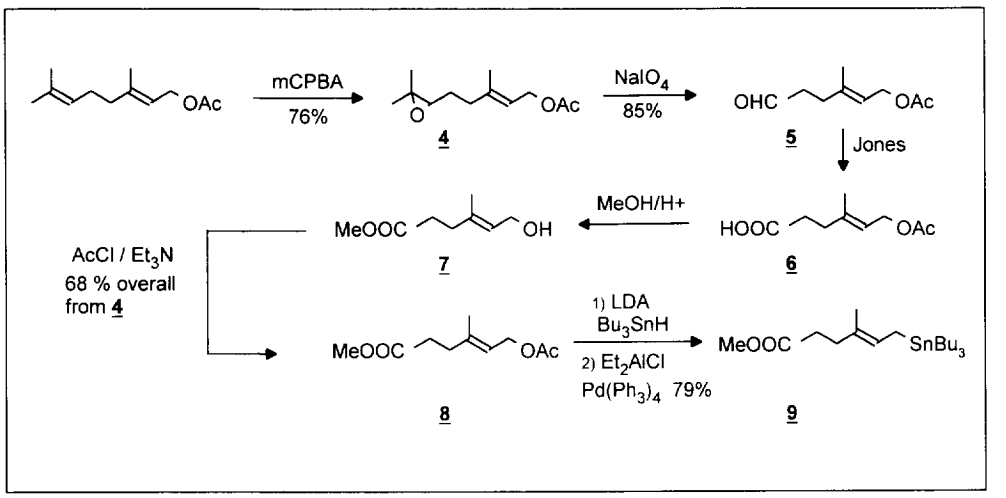


1 2 3

SYNTHESIS OF THE SIDE CHAIN

Geranyl acetate was epoxidized selectively on the terminal double bond with mCPBA in ether at 0°C followed by oxidation with $NaIO_4$ in dioxane at room temperature (**Scheme 1.**). The aldehyde 5 was transformed into the acid 6 with Jones' reagent and esterified with MeOH / H_2SO_4 at room temperature. This led to the allylic alcohol 7 which was reacetylated to give 8. Even though the use of MeOH / H_2SO_4 led to the extra step of

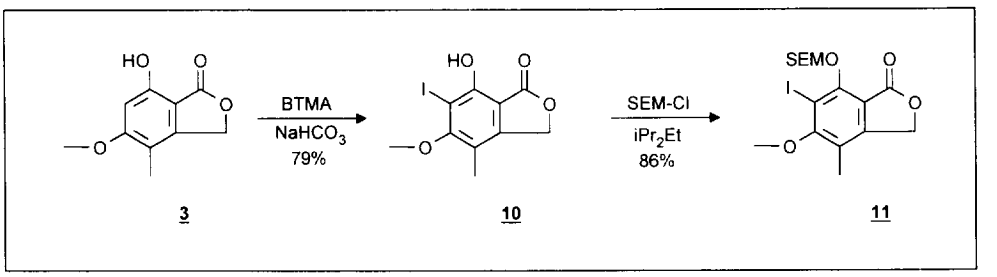
reacylation we found this procedure easier to carry out on 100 g scale than, for example, the use of diazomethane. The allylic acetate **8** was transformed into the corresponding allyltin **9** using the method developed by Trost.[3]



Scheme 1.

FUNCTIONALIZATION OF THE PHTHALIDE RING

The phthalide **3**[4] was iodinated with benzyltrimethyl ammonium dichloroiodate (BTMA)[5] in CH_2Cl_2 in the presence of $NaHCO_3$ at room temperature overnight and the phenol protected with a SEM group to give **11** (**Scheme 2.**).

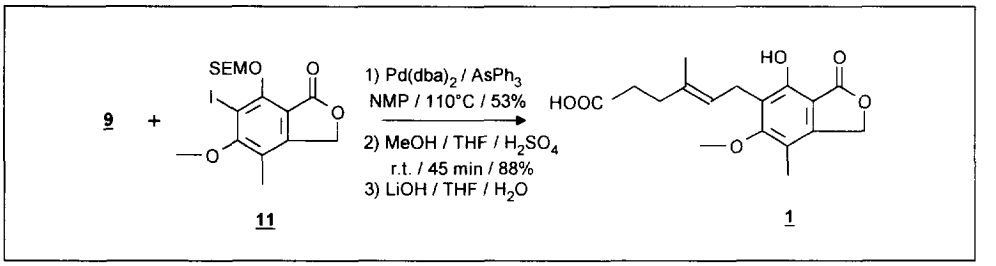


Scheme 2.

COUPLING REACTION

The coupling reaction was accomplished using $AsPh_3/Pd(dba)_2$ in NMP at 110°C (**Scheme 3.**). The use of $AsPh_3/Pd(dba)_2$ was found to be greatly superior to other systems used such as $Pd(PPh_3)_4$, $Pd(dba)_2/P(furyl)_3$, $PdCl_2(PPh_3)_2$, $Pd(OAc)_2/2\ PPh_3$ or $Pd(dba)_2$ alone. 0.3 eq of $Pd(dba)_2$ offers the best compromise in term of

rate of reaction, minimal product degradation and amount of catalyst used. Removal of the SEM group with $MeOH/H_2SO_4$ (88% yield) was found to be quicker and cleaner than TBAF/THF, and saponification of the methyl ester was achieved using LiOH in THF/water. This convergent approach was used successfully for the synthesis of MPA as well as a number of ring modified analogs, the syntheses and biological activities of which will be reported in due course.



Scheme 3.

PROCEDURES

6,7-Epoxygeranyl acetate (4)

Geranyl acetate (100 g, 0.51 mol) in solution in 2.0 l of ether was added via a dropping funnel to a cooled (-30 °C) solution of mCPBA (250 g, ca. 0.72 mol, 50-60% pure from Aldrich) in 2.5 l of ether. Once the addition was complete the temperature was allowed to rise to 0 °C, stirred at this temperature for 6 hrs and placed in a cold room (+ 3 °C) overnight. The reaction mixture was washed with NaOH 1N until pH> 10 then with water until neutral. A final wash with brine, drying over $MgSO_4$ and evaporation of the solvent gave a crude oil. Purification by flash chromatography using 10% ethyl acetate in petroleum ether (40-60°C) gave the desired epoxide **4** (82.2 g, 76 % yield).

E-6-Acetoxy-4-methyl-4-hexenal (5)

The above epoxide (82.2 g, 0.388 mol) was dissolved in dioxane (1.2 l) and treated with $NaIO_4$ (166 g, 0.775 mol) in water (1.7 l) at room temperature and stirred for 24 hrs. The inorganic salt was filtered off and the organic solution diluted with 3 l of ethyl acetate and ether (1:2). The organic phase was washed with water, brine, dried ($MgSO_4$), evaporated and purified by flash chromatography using 10% ethyl acetate in petroleum ether. There were thus obtained 56 g (85 % yield) of the desired aldehyde **5**.[2d]

E-6-Acetoxy-4-methyl-4-hexenoic acid (6)

The above aldehyde (55.9 g, 0.328 mol) was dissolved in acetone (1 l) and Jones reagent (2.7 M) added dropwise until the solution remained orange. The organic phase was diluted with ethyl acetate (3 l) and washed with water until pH ~ 3. A final wash with brine, drying over $MgSO_4$ and evaporation of the solvent gave 53.2 g (87 % yield) of the acid **6** which was used crude for the next step.

Methyl E-6-Hydroxy-4-methyl-4-hexenoate (7)

The carboxylic acid **6** (45 g, 0.242 mol) was dissolved in methanol (500 ml) containing about 25 drops of concentrated sulfuric acid then stirred at room temperature overnight. The reaction mixture was taken-up in ethyl acetate (2 l), washed with water, brine, dried over $MgSO_4$, filtered and concentrated. The crude product **7** (37.6 g) was used for the next step. ^1H NMR (400 MHz, $CDCl_3$) δ 5.41 (m, 1H), 4.13 (d, *J*= 6.9 Hz, 2H), 3.67 (s, 3H), 2.45 (m, 2H), 2.34 (m, 2H), 1.68 (s, 3H).

Methyl E-6-Acetoxy-4-methyl-4-hexenoate (8)

The above ester **7** (37.6 g, 0.238 mol)) was dissolved in CH_2Cl_2 (750 ml), then triethylamine (40 ml, 0.285 mol) and DMAP (0.5 g) were added and the resulting solution was cooled to 0°C. Acetyl chloride was added dropwise and the reaction stirred for 3hrs. The reaction mixture was acidified to pH 2-3 with HCl 1N. The organic phase was washed with water, brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography using 15% ethyl acetate in petroleum ether as eluent to give 36.5 g (77% overall yield from **4**) of **8**. ^1H NMR (400 MHz, $CDCl_3$) δ 5.37 (m, 1H), 4.57 (d, *J*= 7.0 Hz, 2H), 3.67 (s, 3H), 2.46 (m, 2H), 2.36 (m, 2H), 2.05 (s, 3H), 1.72 (s, 3H).

Methyl E-6-Tributylstannyl-4-methyl-4-hexenoate (9)

Distilled THF (100 ml) and $(Bu_3Sn)_2$ (37.8 ml, 75 mmol) were cooled to 0°C while the solution was carefully purged free of oxygen. nBuLi (46.8 ml, 75 mmol, 1.6 M soln in hexanes) was added dropwise (15 min). The resulting clear pale yellow solution was stirred at 0°C for 20 min. Et_2AlCl (41.6 ml, 75 mmol, 1.8 M soln in toluene) was added dropwise (15 min) to the reaction mixture at -78°C and stirred 1 hour at the same temperature. A degassed $Pd(PPh_3)_4$ solution (2.88 g, 2.5 mmol in 70 ml THF) was then added dropwise (10 min) to the reaction mixture at -78°C followed by the allyl acetate solution **8** (10 g, 50 mmol in 10 ml THF). The temperature was then allowed to warm to room temperature over 5 hours. The reaction mixture was placed in a cooling bath at ~ -30°C and a solution of 5 ml NH_4OH (20 %) and 5 ml H_2O was added dropwise. $Al(OH)_3$) was filtered off through Celite. The receiving flask must contain ice and ~ 20 ml NH_4OH (20 %) to avoid decomposition. Extraction with Et_2O followed by washing with water until neutral, brine, drying ($MgSO_4$) and evaporation gave a brown oil. The purification by flash chromatography using 5% ethyl acetate in petroleum ether with 1% Et_3N gave 15.86 g of pure product **9** (73 % yield) which was store under argon at -20 °C. ^1H NMR (400 MHz, DMSOd6) δ 5.36 (m, 1H), 3.66 (s, 3H), 2.37 (m, 2H), 2.28 (m, 2H), 1.64 (d, *J*= 8.8 Hz, 2H), 1.58 (s, 3H), 1.47 (m, 6H), 1.30 (m, 6H), 0.88 (m, 9H), 0.82 (m, 6H).

1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuran (10)

7-hydroxy-5-methoxy-4-methylphtalide (3) (2.0 g, 10.3 mmol) was dissolved in CH_2Cl_2 (100 ml), methanol (40 ml) and benzyl trimethyl ammonium dichloroiodate (BTMA, 3.95 g, 11.3 mmol) was added followed by $NaHCO_3$ (6.0 g, 71 mmol). The reaction mixture was stirred overnight at room temperature. The resulting mixture was diluted with CH_2Cl_2 and acidified to pH ~2 with HCl 1N. The iodine generated was neutralized with solid $Na_2S_2O_3$ and the organic phase was washed with water until pH ~ 6, brine, dried over $MgSO_4$, filtered and concentrated. The product obtained (10, 2.6 g, 79 % yield) was used for the next step. Elem. Anal. Found C 37.7; H 2.9%. Calc. for $C_{10}H_9IO_4$ C 37.5; H 2.83%. ^1H NMR (300 MHz, $DMSOd_6$) δ 10.3 (br s, 1H); 5.28 (s, 2H); 3.76 (s, 3H) and 2.16 (s, 3H).

1,3-Dihydro-4-[2-(trimethylsilyl)ethoxymethoxy]-6-methoxy-7-methyl-3-oxo-5-isobenzofuran (11)

The phenol 10 (698 mg, 2.18 mmol) was dissolved in 10 ml of anhydrous CH_2Cl_2, iPr_2NEt (836 µl, 4.81 mmol) and cooled to -78°C. 2-(Trimethylsilyl)ethoxymethyl chloride (546 mg, 3.28 mmol) was added dropwise then the dry ice bath replaced by an ice bath and the reaction mixture stirred 1.5 hrs. The reaction mixture was diluted with CH_2Cl_2 and adjusted with dil. HCl to pH 5-6. Purification by flash using 15% ethyl acetate in petroleum ether gave 843 mg (86% yield) of the desired product 11. ^1H NMR (400 MHz, $CDCl_3$) δ 5.50 (s, 2H), 5.10 (s, 2H), 3.96 (t, *J*= 7.0 Hz, 2H), 3.82 (s, 3H), 2.23 (s, 3H), 0.94 (t, *J*= 6.9 Hz, 2H), 0.0 (s, 9H).

Methyl E-6-[1,3-Dihydro-4-(2-(trimethylsilyl)ethoxymethoxy)-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoate.

The iodo-aryl 11 (750 mg, 1.67 mmol) was dissolved in 15 ml of anhydrous NMP and sequentially treated with $Pd(dba)_2$ (287 mg, 0.50 mmol) $AsPh_3$ (459 mg, 1.50 mmol) and the organotin 9 (1.08 g, 2.50 mmol). The mixture was carefully purged free of oxygen using alternatively vacuum and argon. The flask was immersed in a pre-heated oil bath (90°C) and the temperature raised to 100 °C for 4hrs. The reaction was monitored by TLC using CH_2Cl_2/petroleum ether/ether (40/50/10) as eluent. The cooled reaction mixture was filtered through a pad of Celite and the filtrate was diluted with ether, washed with water, brine, dried ($MgSO_4$) and evaporated. Purification by preparative HPLC using CH_2Cl_2/ Et_2O/ cyclohexane (60: 1: 39) gave the SEM protected MPA methyl ester (410 mg, 53% yield) as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ 5.38 (s, 2H), 5.24 (m, 1H), 5.11 (s, 2H), 3.83 (m, 2H), 3.75 (s, 3H), 3.60 (s, 3H), 3.44 (d, *J*= 6.6 Hz, 2H), 2.37 (m, 2H), 2.28 (m, 2H), 2.17 (s, 3H), 1.78 (d, *J*= 0.7 Hz, 3H), 0.95 (m, 2H), -0.01 (s, 9H). IR 3000-2850 (broad), 1760, 1740, 1600, 1130, 1070, 960 cm^{-1}. Elem. Anal. Found C 61.88; H 8.00%. Calc. for $C_{24}H_{36}O_7Si$, C 62.04; H 7.81%. CIMS (MNa^+) 487.

Methyl E-6-[1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoate.

The SEM protected MPA methyl ester (400 mg, 0.86 mmol) was dissolved in MeOH (7 ml) and THF (5 ml) and a H_2SO_4 solution (150 µl in 1.5 ml of MeOH) was added at room temperature and stirred for 45 min. The reaction mixture was diluted with ethyl acetate, neutralized to pH 5-6 with $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography using 20% ethyl acetate in pentane gave 253 mg (88% yield) of the MPA methyl ester. Elem. Anal. Found C 64.94; H 7.02%. Calc. for $C_{18}H_{22}O_6$, C 64.66; H 6.63%. ^1H NMR (400 MHz, $CDCl_3$) δ.7.67 (s, 1H), 5.25 (m, 1H), 5.20 (s, 2H), 3.76 (s, 3H), 3.62 (s, 3H), 3.38 (d, *J*= 6.6 Hz, 2H), 2.4 (m, 2H), 2.3 (m, 2H), 2.15 (s, 3H), 1.80 (s, 3H). CIMS (MNa^+) 357.

E-6-[1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoic acid (1)

The ester (245 mg, 0.73 mmol) was dissolved in THF (2 ml) and a solution of $LiOH.H_2O$ (246 mg, 5.8 mmol) in water (2 ml) was added. The reaction mixture was stirred 3 hrs at room temperature then acidified to pH 2 with HCl 1N and stirred at room temperature for 2 hr prior to extraction with ethyl acetate. The organic phase was washed with brine until pH 5, dried ($MgSO_4$), filtered and the solvent evaporated. The crystals were washed twice with ether to give 199 mg (85% yield) of synthetic MPA whose physical characteristics were identical to the natural product purchased from SIGMA.

REFERENCES AND NOTES

1. Biological activity of MPA and closely related analogs see for example: a) Tressler, R. J.; Garvin, L. J.; Slate, D. L. *Int. J. Cancer* **1994**, *57*, 568-573, b) Franklin, T. J.; Morris, W. P. *Adv. Enzyme Regul.* **1994**, *34*, 107-117, c) Hedstrom, L.; Wang, C. C. *Biochemistry* **1990**, *29*, 849-854, d) Sweeney, M. J.; Gerzon, K.; Harris, P. N.; Holmes, R. E.; Poore, G. A.; Williams, R. H. *Cancer Res.* **1972**, *32*, 1795-1802, e) Sweeney, M. J.; Hoffman, D. H.; Esterman, M. A. *Cancer Res.* **1972**, *32*, 1803-1809, f) Franklin, T. J., Cook, J. M. *Biochem. J.*, **1969**, *113*, 515-24.
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4. The phenol was prepared according to reference 2d with the exception that BCl_3 was replaced by $AlCl_3$ (10 eq.) and the reaction mixture stirred 1 hr at room temperature (longer reaction times cause the formation of the diphenol), 81% yield. The dimethoxyphtalide precursor was made as described in the following two references: Logan, W. R.; Newbold, G. T. *J. Chem. Soc.* **1957**, 1946-1951, Trost, B. M.; Rivers, G. T.; Gold, J. M. *J. Org. Chem.* **1980**, *45*, 1835-1838.
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