Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVI, No. 5

Synthesis of Mycophenolate Mofetil-[^{14}C], RS-61443-^{14}C

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SUMMARY

Synthesis of the potent immunosuppressive agent, mycophenolate mofetil (**1**) labelled with carbon-14 is described. Methoxyethoxymethyl (MEM) protected mycophenolate norbromide (**9**) was prepared from unlabelled mycophenolic acid (**2**) using a modified Hunsdiecker reaction. A three step synthesis furnished the title compound, having a specific activity of 53.8 mCi/mmol, in 49.5% overall yield from K^{14}CN.

Key Words: immunosuppressant, Mycophenolate mofetil-^{14}C (MMF-^{14}C), RS-61443-^{14}C, K^{14}CN, mycophenolic acid-^{14}C.

INTRODUCTION

Mycophenolate mofetil (**1**) (MMF, RS-61443), the morpholinoethyl ester of mycophenolic acid (**2**) (1) (MPA), is a potent noncompetitive inhibitor of inosine 5'-monophosphate dehydrogenase (IMPDH) (2-5), the rate-controlling enzyme in the guanosine triphosphate (GTP) "de novo" biosynthesis (6). Since lymphocyte proliferation depends on GTP for protein and nucleic acid synthesis (7), MMF effectively blocks proliferation of mitogen-stimulated T and B lymphocytes and inhibits the generation of antibody and cytotoxic T cells (8).

MMF was developed to enhance the bioavailability of MPA (9). When administered orally, MMF is rapidly absorbed and hydrolyzed to MPA. MPA is subsequently metabolized to its inactive mycophenolate glucuronide conjugate (10). In preclinical trials, MMF has been shown to prolong allograft survival and even to reverse acute allograft rejections in animals (11-13), and is currently in Phase III clinical trials as an immunosuppressant. In order to study its metabolism, tissue absorption and bioavailability, MMF labelled with carbon-14 in the MPA moiety was required.

Contribution # 914 from the Institute of Organic Chemistry, Syntex Discovery Research, Palo Alto, Ca. 94303

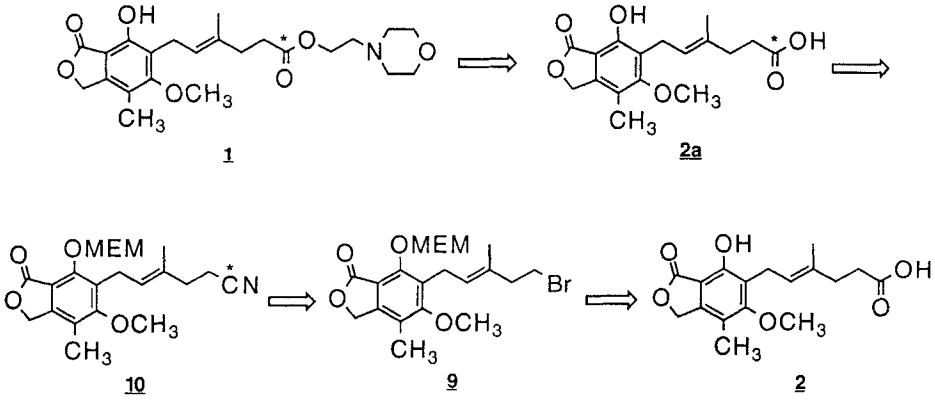
CCC 0362–4803/95/050449–08

Received 11 November, 1994
Revised 12 December, 1994

DISCUSSION

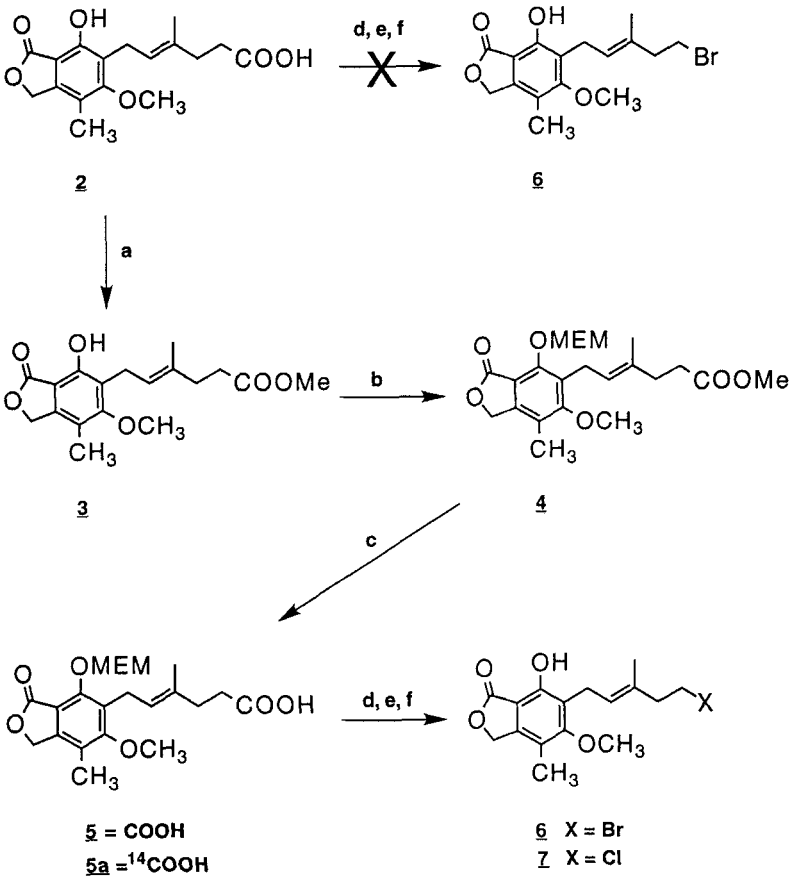
Since MPA is a product of fermentation, intermediates were not readily available. We therefore utilized our previously demonstrated strategy (14), in which an unlabelled product is degraded to furnish a precursor which can be used to reconstruct the product in labelled form. In this particular case, retrosynthetic **Scheme 1** shows our approach. Unlabelled PMA (**2**) is degraded to the norbromide (**9**) via the Hunsdiecker reaction (15). The latter is then homologated with $K^{14}CN$ to regenerate the desired labelled analog (**2a**). Subsequent esterification with hydroxyethylmorpholine would afford the title compound (**1**).

Scheme I



Various modifications of the original Hunsdiecker reaction use metals such as mercury (16), lead (17), and thallium (18) on mostly simple aliphatic acids to produce the corresponding norbromides. Barton et. al. (19) reported a new decarboxylation procedure using thiohydroxamic esters (mixed anhydrides) of aromatic and alkyl carboxylic acids and bromotrichloromethane as the radical chain carrier. Treatment of MPA (**2**) under Barton's condition gave a complex reaction mixture containing no desired norbromide (**6**). We attributed this to the presence of the unprotected phenoxy functionality. In fact, when the phenolic hydroxyl was protected as the MEM ether, moderate yields of (**6**) were obtained, as depicted in **Scheme II**.

Scheme II

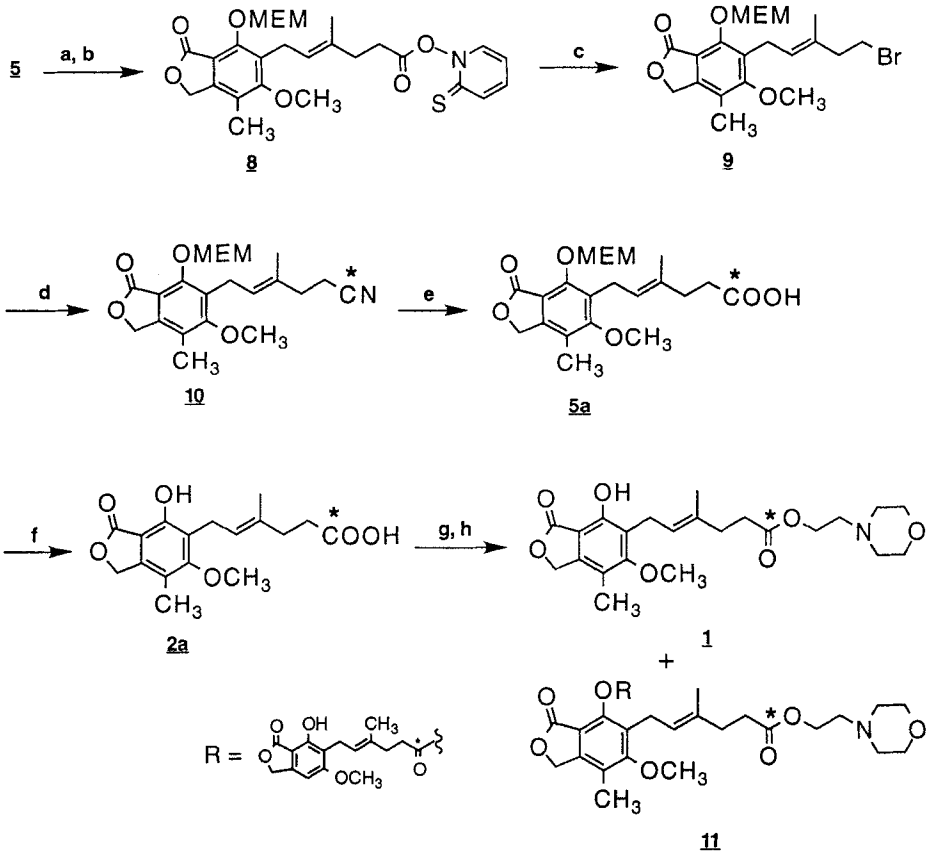


(a) H_2SO_4, MeOH, reflux, 2h; (b) diisopropylethylamine, MEM-Cl, CH_2Cl_2,, 16h; (c) NaOH, MeOH; (d) $(COCl)_2$, DMF, CH_2Cl_2; (e) hydroxypyridine-2-thione sodium salt, DMAP, toluene; (f) $CBrCl_3$.

MPA (2) was esterified with H_2SO_4 in MeOH. The resulting methyl ester (3) was dissolved in CH_2Cl_2 and treated with diisopropylethylamine followed by MEM-Cl to give the MEM-protected methyl ester (4). Saponification of (4) with 0.1N NaOH furnished the MPA MEM ether (5) in 90% overall yield from (2). Treatment of (5) under Barton's condition gave yields ranging from 10-80 % of varying mixtures of the norbromide (6) and the norchloride (7). Furthermore, TLC analysis showed that the acid chloride forming step resulted in concomitant loss of the MEM group.

These problems prompted us to use a modified version of Barton's method developed in our laboratory (20), as depicted in **Scheme III**.

Scheme III



(a) DCC, CH_2Cl_2; (b) hydroxypyridine-2-thiol, CH_2Cl_2; (c) $CBrCl_3$, hv; (d) $K^{14}CN$, DMSO, 100 °C, 3.5h; (e) KOH, $MeOCH_2CH_2OH$, reflux, 16h; (f) HCl, THF, reflux, 75 min.; (g) $(COCl)_2$, CH_2Cl_2; (h) hydroxyethyl-morpholine, CH_2Cl_2.

Thus, MPA MEM ether (5) was stirred with DCC and in CH_2Cl_2 at ambient temperature for 15 min followed by addition of hydroxypyridine-2-thiol. This mixture was stirred for an additional 30 min then filtered. To the filtrate containing the intermediate thione ester (8) was added $CBrCl_3$ and the mixture was exposed to direct sunlight for 10 min to give the desired MEM-protected norbromide (9) in 43% yield from (5).

The norbromide (**9**) was treated with K^{14}CN in DMSO to give the labelled nitrile (**10**) in 67% yield. Hydrolysis with 20% KOH in refluxing methoxyethanol for 16h afforded ^{14}C-MPA MEM ether (**5a**) in 89% isolated yield. The MEM protecting group was removed with 15% HCl in refluxing THF for 75 min to give a quantitative yield of ^{14}C-MPA (**2a**). The final esterification step was effected by converting ^{14}C-MPA (**2a**) to its acid chloride with oxalyl chloride containing a catalytic amount of DMF in CH_2Cl_2. This was added directly to an ice cooled solution of hydroxyethylmorpholine in CH_2Cl_2 and stirred for 45 min at 0 °C. Warming to ambient temperature gave a 90:10 mixture of the title compound (**1**) and a less polar by-product (**11**), respectively. By-product (**11**) is believed to be the diester resulting from the addition of the acid chloride of MPA to the phenoxy moiety of (**1**). The identity of (**11**) was indirectly demonstrated by hydrolysis back to MPA (**2**) with 1 N NaOH at reflux for 16h (95% yield), based by HPLC analysis. It is interesting to note that if the acid chloride of MPA was added to hydroxyethyl-morpholine and stirred at ambient temperature rather than at 0°, then the product ratio of (**2**) and (**11**) worsen to 55:45, respectively. The crude product was purified by radial chromatography to give mycophenolate mofetil-[^{14}C] (**1**) in 83% yield (49.5% overall yield from K^{14}CN), having a specific activity of 53.8 mCi/mmol by UV.

This synthesis demonstrates a general method of preparing a labelled carboxylic acid from its unlabelled analog, when precursors are not readily available. Furthermore, this method utilizes K^{14}CN, a relatively inexpensive source of C-14, and affords the desired product in very good radiochemical yield.

EXPERIMENTAL

Base free K^{14}CN was prepared in-house from Ba$^{14}CO_3$. All reagents were purchased from Aldrich Chemical Company and were used without purification. Solvents were HPLC grade. Radiochromatography was performed on a Bioscan 200 scanner. Radioassays were obtained from a Packard 4000 liquid scintillation counter. HPLC analyses were obtained on a Beckman System Gold gradient system equipped with 166 UV detector and 171 radioactivity flow detector. UV spectra were obtained on a Hitachi UV-265 spectrophotometer. NMR spectra were obtained on a Bruker 300 MHz spectrometer. MS spectra were obtained on a Finnigan-MAT 8230 spectrometer.

Mycophenolic acid MEM ether (5)

To mycophenolic acid (**2**) (10 gm, 31.25 mmol) in MeOH (50 ml) was added a few drops of conc H_2SO_4 and the mixture was heated at reflux for 2 h. The crude reaction mixture was concentrated to dryness, and the solid product dissolved in EtOAc (100 ml). The organic layer was washed with $NaHCO_3$(50 ml), H_2O (50 ml), brine (50 ml), and dried ($MgSO_4$), then concentrated to give a quantitative yield of mycophenolic acid methyl ester (**3**). This material was dissolved in CH_2Cl_2 (75 ml) and treated with diisopropylethylamine (8 ml, 45.92 mmol) then MEM-Cl (5 ml, 43.79 mmol). The mixture was stirred at ambient temperature for 16 h. The crude reaction mixture was washed with 10% HCl (50 ml), saturated $NaHCO_3$ (50 ml), H_2O (50 ml), brine (50 ml), and dried ($MgSO_4$). Concentration of the organic phase gave a quantitative yield of MEM-protected

mycophenolic acid methyl ester (**4**) as a yellow oil. Saponification with NaOH (0.1N, 50 ml) in MeOH (50 ml) followed by acidification with conc HCl and extraction with EtOAc (3x50 ml) afforded 11.45 gm of the mycophenolic acid MEM ether (**5**) (90% overall yield). This material was used as is in the next step.

TLC: silica gel, R_f (hexane-EtOAc-HOAc, 1:1:1%) 0.4; ^1H NMR ($CDCl_3$, 300MHz) δ 5.41(2H, s, OCH_2O), 5.25 (1H, t, C=CH-), 5.13 (2H, s, OCH_2Ph), 3.6-3.9 (4H, m, OCH_2CH_2O), 3.76 (3H, s, $PhOCH_3$), 3.59 (2H, d, $PhCH_2C$=), 3.38 (3H, s, OCH_3), 2.3 (4H, m, CH_2CH_2COOH), 2.18 (3H, s, $PhCH_3$), 1.8 (3H, s, C=C-CH_3); MS 408(M+), 332, 319, 247, 207. Anal. Calcd for $C_{21}H_{28}O_8$: C, 61.75; H, 6.91. Found: C, 61.97; H, 6.95.

Mycophenolate norbromide (9)

To 1-hydroxypyridine-2-thione, sodium salt (10 gm, 66.7 mmol) suspended over CH_2Cl_2 (100 ml) was added Dowex cation exchange resin (90 meq, 50W-X8, 20-50 mesh) and the mixture was stirred for 10 min. The resin was filtered and the resulting 1-hydroxypyridine-2-thiol (8.1 gm, 96%) was used as is for the next step. To mycophenolic acid MEM ether (**5**) (2.04 gm, 5 mmol) dissolved in CH_2Cl_2 (46 ml) was added DCC (1.23 gm, 6 mmol) under N_2 and the mixture was stirred at ambient temperature for 15 min. Hydroxypyridine-2-thiol (700 mg, 5.5 mmol) prepared above was then added in portions over several minutes and the mixture was stirred at ambient temperature for another 30 min. The solid by-products were filtered and $CBrCl_3$ (5 ml, 50 mmol) was added to the filtrate containing intermediate (**8**). This mixture was then exposed directly to sunlight until the yellow color faded. The crude product was concentrated to give, after chromatographic purification, 770 mg of mycophenolate norbromide (**9**) (43%).

TLC: silica gel, R_f (hexane-EtOAc, 1:1) 0.6; ^1H NMR ($CDCl_3$, 300MHz) δ 5.43 (2H, s, OCH_2O), 5.3 (1H, t, C=CH-), 5.13 (2H, s, OCH_2Ph), 3.75 (4H, m, OCH_2CH_2O), 3.78 (3H, s, $PhOCH_3$), 3.47 (2H, d, $PhCH_2C$=), 3.45 (2H, t, -CH_2Br), 3.38 (3H, s, OCH_3), 2.5 (2H, t, =C-CH_2), 2.18 (3H, s, $PhCH_3$), 1.8 (3H, s, C=C-CH_3); MS 444, 442 (M+).

Mycophenolate nitrile-[^{14}C] MEM ether (10)

K^{14}CN (84 mCi, 1.43 mmol; 59 mCi/mmol) in DMSO (10 ml) was heated at 100°C for 1 h, then a solution of mycophenolate norbromide (**9**) (678 mg, 1.53 mmol) in DMSO (10 ml) was added and the mixture heated at 100°C for 3.5 h. The reaction was cooled to ambient temperature and then partitioned between H_2O (50 ml) and EtOAc (50 ml). The aqueous phase was re-extracted with EtOAc (2X50 ml). The combined organic phase was washed with 1:1 H_2O-brine (50 ml), brine (50 ml), and dried (Na_2SO_4) to give, after chromatographic purification, 56.6 mCi of pure mycophenolate nitrile-[^{14}C] MEM ether (**10**) (67%).

Radio-tlc: silica gel, R_f (hexane-EtOAc, 1:1) 0.5.

Mycophenolic acid-[^{14}C] MEM ether (5a)

To mycophenolate nitrile-[^{14}C] MEM ether (**10**) (54.55 mCi, 0.92 mmol) in methoxyethanol (50 ml) was added 20% KOH (10 ml, 35.7 mmol) and the mixture was heated at reflux for 16 h, then cooled to ambient temperature. The reaction mixture was partitioned between EtOAc (50 ml) and saturated $NaHCO_3$ (50 ml). The aqueous phase was acidified with conc HCl and the product was

extracted into EtOAc (3x50 ml). The combined organic phase was washed with H_2O (50 ml), brine (50 ml), and dried (Na_2SO_4) to give, after chromatographic purification, 48.7 mCi of pure mycophenolic acid-[^{14}C] MEM ether (**5a**) (89%).
Radio-tlc: silica gel, R_f (hexane-EtOAc-HOAc, 1:1:1%) 0.4.

Mycophenolic acid-[^{14}C] (2a)
To mycophenolic acid-[^{14}C] MEM ether (**5a**) (48.7 mCi, 0.83 mmol) in THF (60 ml) was added 15% HCl (35 ml) and the mixture was heated at reflux for 75 min, then cooled to ambient temperature. THF was removed by rotary evaporation and the product was extracted into EtOAc (3x50ml). The combined organic phase was washed with H_2O (50 ml), brine (50 ml) and dried (Na_2SO_4) to afford quantitative yield of pure mycophenolic acid-[^{14}C] (**2a**).
Radio-tlc: silica gel, R_f (hexane-EtOAc-HOAc, 1:1:1%) 0.5.

Mycophenolate-[^{14}C]-mofetil (1)
To mycophenolic acid-[^{14}C] (**2a**) (30 mCi, 0.52 mmol) in CH_2Cl_2 (20 ml) were added oxalyl chloride (0.45 ml, 5.2 mmol) and cat DMF. The mixture was stirred at ambient temperature for 30 min. Excess oxalyl chloride was removed by rotary evaporation and the residue was reconstituted with CH_2Cl_2 (20 ml). This was then added dropwise to an ice cooled solution of hydroxyethylmorpholine (0.3 ml, 2.5 mmol) in CH_2Cl_2 (20 ml) and the mixture was stirred at 0 °C for 30-45 min before warming to ambient temperature. The reaction was diluted with CH_2Cl_2 (50 ml) then washed with $NaHCO_3$ (50 ml), H_2O, brine (50 ml), and dried (Na_2SO_4) to afford, after chromatographic purification, 25 mCi of pure title compound (**1**) (83%), having a specific activity of 53.8 mCi/mmol and 3 mCi of (**11**).
Radio-tlc: silica gel, R_f **1**(CH_2Cl_2-MeOH, 95:5) 0.5, **11** 0.6; Reverse phase C-18, R_f **1**(MeOH-H_2O, 4:1) 0.4, **11** 0.5; HPLC: Vydac 201TP54, RP C-18, 4.6mm x 250mm, (25:75) CH_3CN-0.03M triethylammonium phosphate buffer, pH=3; 249 nm, 1 ml/min, R_t (13.1 min), UV (ethanol): ε8200, λ_{max} 249nm .

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Steve de Keczer (Radiochemistry Department, Institute of Organic Chemistry, Syntex Discovery Research) for the preparation of K^{14}CN.

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