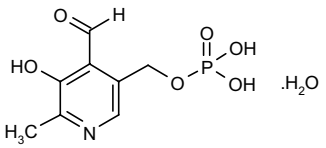
Drugs of the Future 2006, 31(11): 953-958

CCC: 0377-8282/2006
DOI: 10.1358/dof.2006.031.11.1037129

# MC-1/MC-4232

**MC-1** *Cardioprotective Agent*

Phosphoric acid (4-formyl-5-hydroxy-6-methylpyridin-3-yl)methyl ester hydrate
Pyridoxal-5'-phosphate monohydrate



$C_8H_{12}NO_7P$
Mol wt: 265.1572

EN: 287697

**MC-4232** *Treatment of Hypertension*

Combination of MC-1 and lisinopril
EN: 341337

**Abstract**

MC-1 is a naturally occurring small molecule, a metabolite of vitamin $B_6$, that reduces the damage to the heart caused by ischemia and/or ischemia-reperfusion injury, and has very low toxicity. The safety and efficacy of MC-1 in the treatment of various cardiovascular diseases have been demonstrated in both preclinical and clinical studies. MC-4232 is a combination of MC-1 and lisinopril. Preclinical animal studies and clinical studies in patients with co-existing type 2 diabetes and hypertension have demonstrated a promising safety profile and efficacy for MC-4232 in reducing blood pressure and controlling metabolic dysfunction. Based on the positive results obtained from phase II clinical studies, both MC-1 and MC-4232 are scheduled to enter phase III studies.

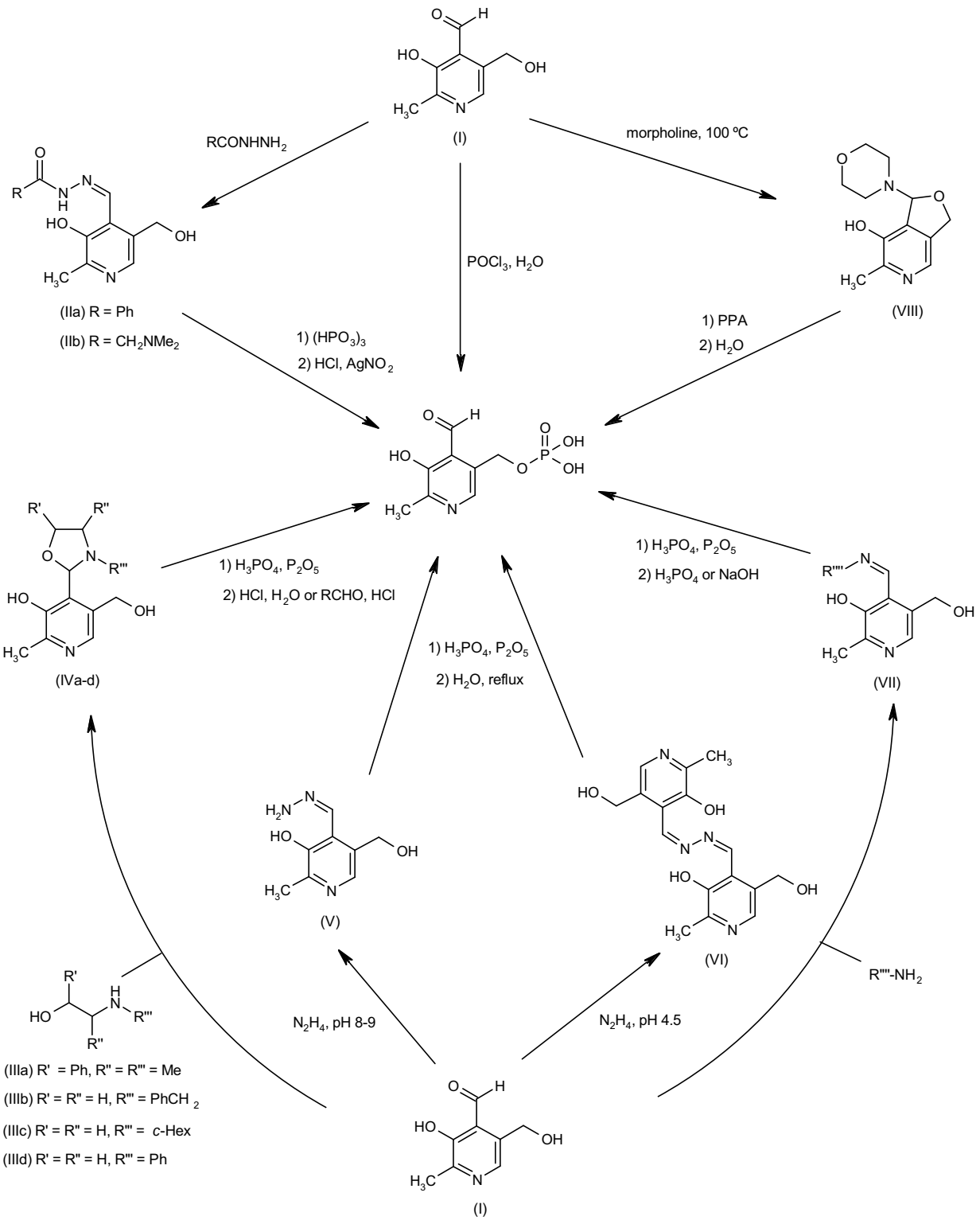
## Synthesis

MC-1 can be synthesized by several different ways:

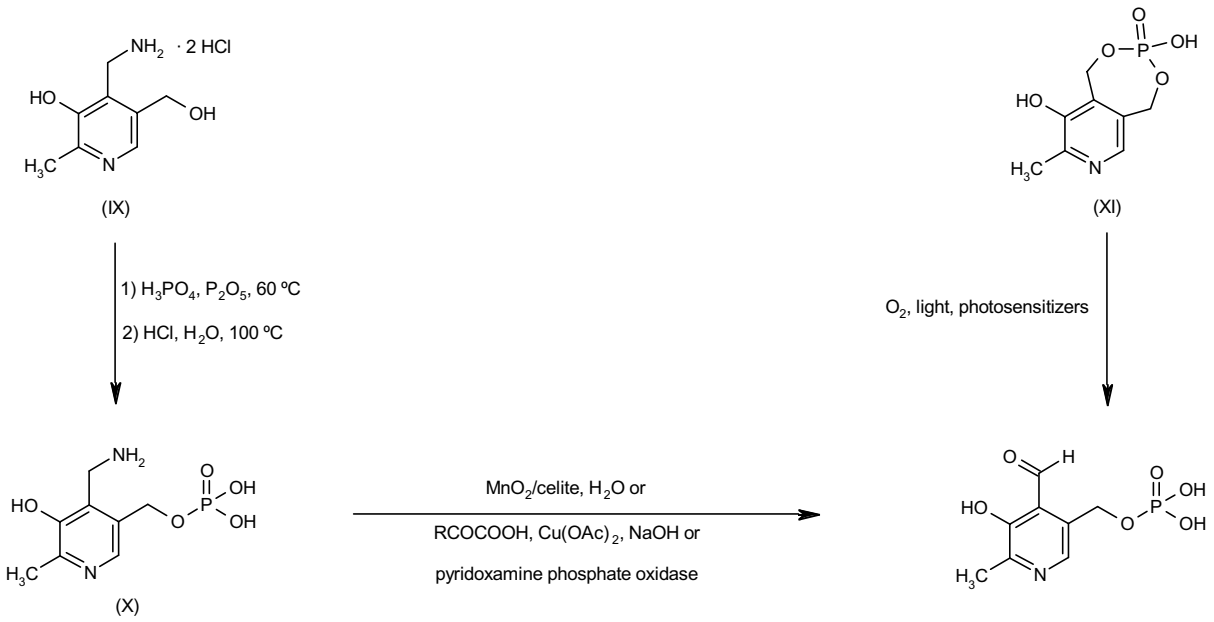
MC-1 can be synthesized in low yields by direct phosphorylation of pyridoxal (I) with $POCl_3$ in aqueous solution (1). Several improved methods have been disclosed involving the protection of the aldehyde function of pyridoxal before phosphorylation. The pyridoxal hydrazones (IIa,b), obtained by condensation of pyridoxal (I) with either benzoylhydrazine or *N,N*-dimethylglycylhydrazine, are phosphorylated with *meta*-phosphoric acid, followed by hydrolysis with HCl and $AgNO_2$, to provide the target phosphate (2, 3). Alternatively, pyridoxal (I) is converted to the corresponding oxazolidines (IVa-d) by condensation with different amino alcohols, including ephedrine (IIIa), 2-benzylaminoethanol (IIIb), 2-cyclohexylaminoethanol (IIIc) and *N*-(2-hydroxyethyl)aniline (IIId). The phosphorylation of (IV) by means of polyphosphoric acid, generated from $H_3PO_4$ and $P_2O_5$, produces the corresponding phosphate esters, which are finally hydrolyzed to pyridoxal phosphate with aqueous HCl (4), or via formation of an intermediate cyclic acetal with several aldehydes (5). The hydrazone (V) and azine (VI) of pyridoxal are obtained by treatment of (I) with an excess of hydrazine at pH 8-9 or by treatment with the equivalent amount of hydrazine in NaOAc buffer at pH 4.5, respectively (6). Phosphorylation of pyridoxal hydrazone (V) by means of $H_3PO_4/P_2O_5$, followed by aqueous hydrolysis of any existing polyphosphate ester, leads to pyridoxal phosphate hydrazone, which is converted to the title compound by treatment with isoamyl nitrite and HCl (7). Similarly, the dimeric azine (VI) is phosphorylated with $H_3PO_4/P_2O_5$ to yield, after hydrolysis of the polyphosphate groups, the azine bisphosphate, which is finally subjected to diazoic decomposition to give the target pyridoxal phosphate (8). The condensation of pyridoxal (I) with a variety of primary aliphatic and aromatic amines, optionally in the presence of metallic salts such as $NiCl_2$, gives the corresponding imines (VII) (9-11). Phosphorylation of the pyridoxal imines (VII) with $H_3PO_4/P_2O_5$ as in the above methods gives the corresponding phosphates, which finally undergo imine hydrolysis under acidic or alkaline conditions (10-13). The protection of pyridoxal (I) with alicyclic secondary amines, such as morpholine, gives the bicyclic furopyridine derivative (VIII). Transformation of (VIII) to the desired pyridoxal phosphate is then accomplished by heating with polyphosphoric acid, followed by hydrolysis of the reaction mixture by the addition of water (14). Scheme 1.

Y. Wang. 8106 Runnymeade Dr., Frederick, MD 21702, USA. J. Bolós, N. Serradell. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

**Scheme 1: Synthesis of MC-1**



**Scheme 2: Synthesis of MC-1**

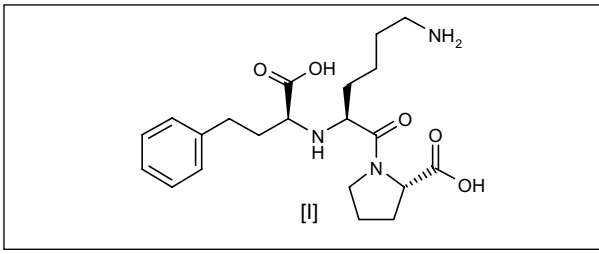



Pyridoxal phosphate can also be obtained via phosphorylation of different pyridoxal precursors. Treatment of pyridoxamine (IX) with $H_3PO_4/P_2O_5$, followed by acidic hydrolysis, provides pyridoxamine phosphate (X) (15, 16). The subsequent oxidation of amine (X) to the target aldehyde has been reported under a variety of conditions, including chemical oxidation with $MnO_2$ (15, 16), transamination with $\alpha$-ketoacids, including pyruvic acid, in the presence of cupric and other metal ions (17), and enzymatic oxidation with cell-free extracts from bacteria such as *Alcaligenes faecalis* (18). Alternatively, pyridoxal phosphate can be prepared by photo-oxidation of pyridoxine-4,5-cyclic phosphate (XI) in the presence of a suitable photosensitizer, including riboflavin, flavin monophosphate, 6,7,8-trimethyllumazine or lumiflavine, and amines such as aniline (19). Scheme 2.

In a different method involving pyridine ring construction, the Diels-Alder cycloaddition of 5-ethoxy-4-methyloxazole (XII) with 2,5-dimethoxy-2,5-dihydrofuran (XIII) provides the epoxy-furopyridine adduct (XIV) as a mixture of *endo*- and *exo*-isomers. Subsequent treatment of (XIV) with methanolic KOH leads to the furopyridine (XV), which is subjected to acidic hydrolysis to afford the pyridine dialdehyde cyclic monohydrate (XVI). Reaction of (XVI) with a 2-mercaptoamine such as 2-cyclohexylaminoethanethiol (XVII) in the presence of HCl yields the 4-(thiazolidinyl)pyridine-3-carbaldehyde (XVIII), which is reduced to the alcohol (XIX) utilizing $NaBH_4$. Finally, phosphorylation of (XIX), followed by thiazolidine ring hydrolysis, gives the desired pyridoxal phosphate (20). Scheme 3.

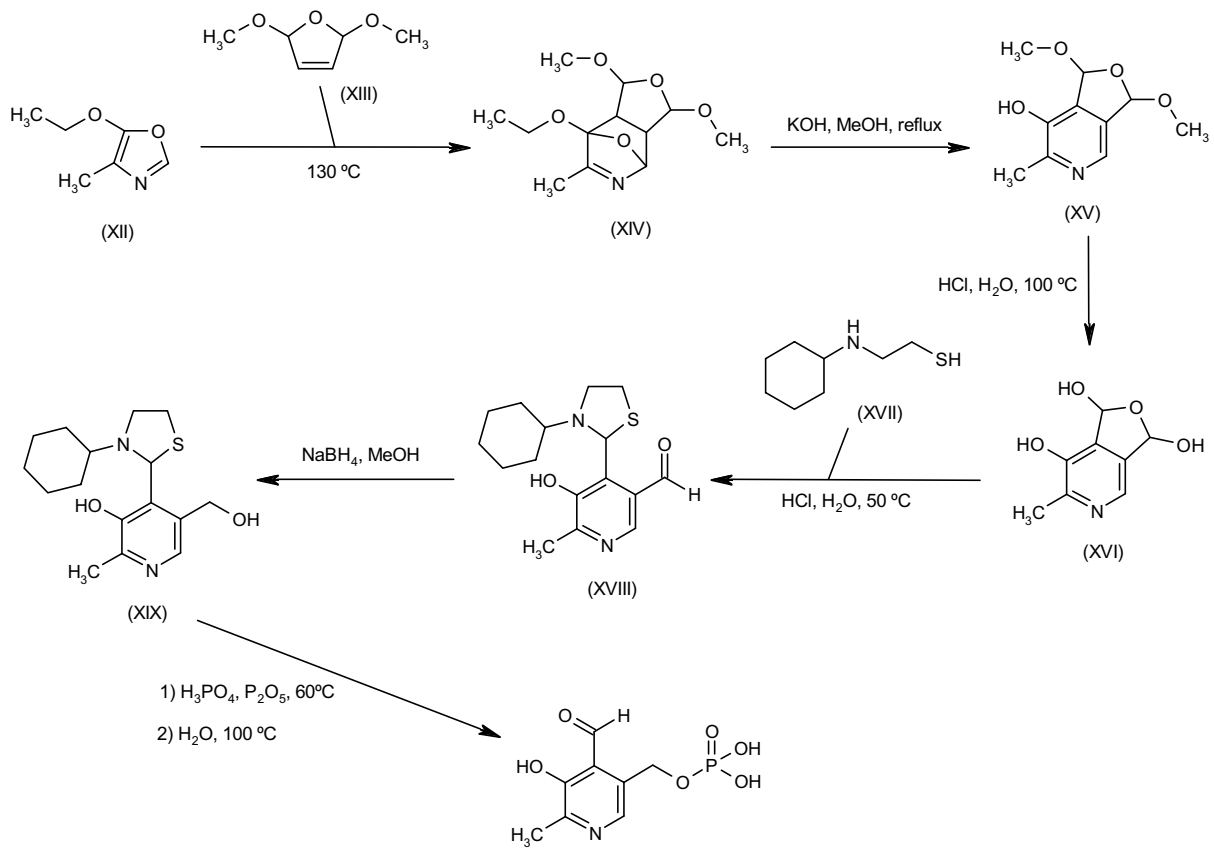
## Background

MC-1, a metabolite of vitamin $B_6$, has the ability to reduce the amount of damage to the heart caused by ischemia and/or ischemia-reperfusion injury by protecting cardiomyocytes. Medicure is developing MC-1 as a treatment to reduce cardiovascular events associated with ischemia and/or ischemia-reperfusion injury in patients undergoing coronary artery bypass graft (CABG) surgery, percutaneous coronary intervention (PCI) and acute coronary syndrome (ACS). The company is also developing MC-4232, a combination of MC-1 and the angiotensin-converting enzyme (ACE) inhibitor lisinopril [I], as a candidate for the treatment of diabetic patients with hypertension. Preclinical and initial clinical studies of MC-1 and MC-4232 have shown positive results and pivotal phase III trials are planned. MC-1 was granted fast track designation by the FDA for reducing cardio- and cerebrovascular events associated with ischemia or ischemic reperfusion injury in patients undergoing PCI or CABG surgery, or those with ACS (21-24).




[I]

**Scheme 3: Synthesis of MC-1**




## Preclinical Pharmacology

The triglyceride-lowering effects of MC-1 were evaluated in a study in animals fed a high-cholesterol diet to increase the levels of triglycerides, total cholesterol and LDL cholesterol. Compared with controls, treatment with MC-1 at a dose of 10 mg/kg for 7 weeks led to a 72% reduction in triglyceride levels. Moreover, MC-1 demonstrated an improvement in triglyceride-lowering effect compared with an approved, widely prescribed triglyceride-reducing agent (25).

In another preclinical study, MC-1 demonstrated greater beneficial effects than four available cardiovascular drugs in the treatment of animals with ischemic heart disease. The study evaluated MC-1 in combination and comparison with aspirin and with an ACE inhibitor, a β-blocker, and a calcium channel blocker. MC-1 demonstrated at least equivalent efficacy in terms of the size of scar formation, mortality and improvement in heart function. Additive effects were observed for the combinations (26).

The protective effects of MC-1 alone and in combination with tissue plasminogen activator (tPA) in focal ischemic brain injury in rats were also examined. The study demonstrated that MC-1 was effective in protecting the brain after ischemic injury at doses of 40-120 mg/kg by infusion starting 1 h after injury, and a neuroprotective effect was still evident even when MC-1 was administered up to 6 h after injury (27).

## Clinical Studies

To evaluate the effect of MC-1 as a cardioprotective agent in high-risk patients undergoing elective PCI, the MEND-1 (MC-1 to Eliminate Necrosis and Damage) trial was conducted in both Canada and the U.S. A total of 60 patients who underwent PCI were randomized to MC-1 or placebo. MC-1 10 mg/kg was administered at least 4 h before PCI, and then at a dose of 5 mg/kg twice daily for 14 days. The primary objective of the study was to evaluate the feasibility of MC-1 as a cardioprotective agent in high-risk elective PCI. The primary endpoint of the study was defined as infarct size during the surgery determined by the amount of CK-MB release over 24 h after PCI. The secondary endpoints included myocardial ischemia mea-

sured by continuous S-T segment electrocardiographic monitoring, peak periprocedural CK-MB through 24 h and clinical tolerability and safety. The results indicated that the study met both the primary and secondary endpoints. Periprocedural CK-MB was reduced from 32.9 ng/ml in the placebo group to 18.6 ng/ml in MC-1 group. The occurrence of 30-day nonfatal acute myocardial infarction (AMI) did not differ between groups. Major bleeding (2.8% on MC-1 *vs.* 10.5% on placebo) did not differ significantly between groups (28, 29).

A double-blind, randomized, placebo-controlled, parallel-group phase II trial (MEND-CABG) was then conducted to evaluate the cardioprotective and neuroprotective effects of MC-1 (250 and 750 mg/day) in high-risk coronary artery disease patients undergoing CABG surgery. The trial enrolled 901 patients who underwent CABG surgery at 42 sites throughout Canada and the U.S. The primary endpoint of the trial was a reduction in the combined incidence of death, nonfatal myocardial infarction and nonfatal stroke up to postoperative day (POD) 30. The secondary efficacy endpoints of the trial included the effect of MC-1 at POD 90 on the same composite of events, cardiac tissue damage as determined by CK-MB and neurological function. Results demonstrated that treatment with 250 mg of MC-1 led to a 37.2% reduction in the composite of death, nonfatal myocardial infarction and nonfatal stroke. The reduction in the composite endpoint was driven by a substantial decrease in the incidence of nonfatal myocardial infarction, most notably a 46.9% reduction in nonfatal myocardial infarction with this dose of MC-1 *versus* placebo. The beneficial effect was maintained through day 90 and the treatment was well tolerated. The lower dose was more effective than the higher dose (23, 30).

The phase II MATCHED (MC-1 and ACE Therapeutic Combination for Hypertensive Diabetics) study, a randomized, double-blind, placebo-controlled, crossover trial, was conducted in 160 patients with co-existing type 2 diabetes and hypertension (mean daytime ambulatory systolic blood pressure = 135 mmHg or more) who received MC-1 (100, 300 or 100 mg b.i.d.) + lisinopril (20 mg once daily) (MC-4232) or placebo for 8 weeks, followed by 8 weeks' treatment with placebo or MC-1 alone, or the combination for 8 weeks followed by placebo or lisinopril alone for 8 weeks. The primary endpoints of the study included metabolic function and blood pressure. The study demonstrated the positive clinical effects of MC-4232 (MC-1 300 mg + lisinopril) on blood pressure, including both systolic and diastolic measurements, and metabolic dysfunction, including glycemic control measured by fasting serum glucose and glycosylated hemoglobin (HbA1c), as well as lipid control. The combination was more effective than lisinopril or MC-1 alone in reducing blood pressure. Treatment-related adverse events were uncommon and similar to on placebo (31-33).

## Source

Medicure, Inc. (CA).

## References


1. Gunsalus, I.C., Umbreit, W.W., Bellamy, W.D., Foust, C.E. *Some properties of synthetic codecarboxylase*. J Biol Chem 1945, 161(2): 743-4 (Letters to the Editor).

2. Karrer, P., Viscontini, M. (Hoffmann-La Roche, Inc.). *Manufacture of pyridoxal-5'-phosphoric acid ester and salts thereof*. US 2703323.

3. Roche Products, Ltd. *Process for the manufacture of pyridoxal-5'-phosphoric acid ester and salts thereof*. GB 711442.

4. Schorre, G. (E. Merck AG). *Process for the production of pyridoxal-5'-orthophosphoric acid ester*. GB 880595, US 3124587.

5. E. Merck AG. *Pyridoxal-5'-phosphates and preparation thereof*. GB 1111876.

6. McCormick, D.B., Snell, E.E. *Pyridoxal phosphokinases. II. Effects of inhibitors*. J Biol Chem 1961, 236(7): 2085-8.

7. Okumura, K., Moritani, T. (Tanabe Seiyaku Co., Ltd.). *Process for preparing pyridoxal-5'-ortophosphate*. FR 1477940, GB 1076910.

8. Okumura, K., Nishihara, T. (Tanabe Seiyaku Co., Ltd.). *Process for preparing pyridoxal-5'-orthophosphate*. FR 1473663, US 3404154.

9. Heyl, D., Luz, E., Harris, S.A., Folkers, K. *Chemistry of vitamin B6. VII. Pyridoxylidene- and pyridoxylamines*. J Am Chem Soc 1948, 70(11): 3669-71.

10. Iwanami, M., Numata, T., Murakami, M. *A new synthesis of pyridoxal-5-phosphate*. Bull Chem Soc Jpn 1968, 41(1): 161-5.

11. Dainippon Pharmaceutical Co., Ltd. *Process for preparing pyridoxal-5'-monophosphate and intermediates and new products thus obtained*. FR 2003702.

12. Vila Busquets, P. *Process for the preparation of pyridoxal phosphate*. ES 421396.

13. Yamanouchi Pharmaceutical Co., Ltd. *Improvements relating to the production of pyridoxal phosphate*. GB 1074885.

14. Nakagawa, K., Yoshimura, I., Sueda, N., Fukawa, H. *A novel synthesis of pyridoxal-5'-phosphate*. Agric Biol Chem 1977, 41(8): 1431-3.

15. Wilson, A.N., Harris, S.A. *Phosphates of the vitamin B6 group. V. A synthesis of codecarboxylase*. J Am Chem Soc 1951, 73(10): 4693-4.

16. Peterson, E.A., Sober, H.A. *Preparation of crystalline phosphorylated derivatives of vitamin $B_6$*. J Am Chem Soc 1954, 76(1): 169-75.

17. Roche Products, Ltd. *A process for the manufacture of pyridoxal phosphate*. GB 749800.

18. Yamamoto, S., Tochikura, T., Ogata, K. *Studies on vitamin $B_6$ metabolism in microorganisms. Part I. Pyridoxine phosphate and pyridoxamine phosphate oxidation (1). Distribution in bacteria and isolation of oxidized product*. Agric Biol Chem 1965, 29(3): 200-7.

19. Kyowa Hakko Kogyo Kabushiki Kaisha. *Process for preparing pyridoxal-5-phosphate*. GB 1166425.

20. Daiichi Seiyaku Co., Ltd. *Process for manufacturing pyridoxal-5-phosphate*. GB 1164330.

21. *Medicure acquires Aggrastat from MGI Pharma*. DailyDrugNews.com August 16, 2006.

22. *Single confirmatory phase III study planned for MC-1*. DailyDrugNews.com April 19, 2006.

23. *Positive results disclosed from MEND-CABG study of MC-1*. DailyDrugNews.com December 9, 2005.

24. *Medicure receives FDA fast track designation for MC-1*. DailyDrugNews.com September 22, 2005.

25. *MC-1 shows preclinical potential for hypertriglyceridemia*. DailyDrugNews.com May 10, 2005.

26. *Medicure's MC-1 shows good results in ischemia models*. DailyDrugNews.com March 20, 2000.

27. Wang, C.X., Yang, T., Noor, R., Shuaib, A. *Role of MC-1 alone and in combination with tissue plasminogen activator in focal ischemic brain injury in rats*. J. Neurosurg 2005, 103(1): 165-9.

28. *Phase II trial of MC-1 meets primary and secondary endpoints*. DailyDrugNews.com January 16, 2003.

29. Kandzari, D.E., Labinaz, M., Canlor, W.J. et al. *Reduction of myocardial ischemic injury following coronary intervention (The MC-1 to Eliminate Necrosis and Damage Trial)*. Am J Cardiol 2003, 92(6): 660-4.

30. *Medicure reports Q3 2006 R&D highlights*. Medicure Press Release 2006, April 13.

31. Lefebvre, J., Poirier, L., Stewart, N., Lacourciere, Y. *The combination of MC-1 and lisinopril has beneficial effects on carbohydrate and lipid metabolism in hypertensives with type 2 diabetes mellitus*. J Clin Hypertension (Greenwich) 2006, 8(5, Suppl. A): Abst P-107.

32. Lacourciere, Y., Poirier, L., Lefebvre, J., Stewart, N. *Evaluation of the antihypertensive effects of MC-1 alone and in combination with lisinopril on ambulatory BP in hypertensive patients with type 2 diabetes mellitus*. J Clin Hypertension (Greenwich) 2006, 8(5, Suppl. A): Abst P-205.

33. Lacourciere, Y., Lefebvre, J., Poirier, L., Stewart, N., Zettler, M. *Novel combination of MC-1 and lisinopril lowers blood pressure and CRP levels and improves metabolic function in hypertensive diabetic patients*. 66th Annu Meet Sci Sess Am Diabetes Assoc (ADA) (June 9-13, Washington, D.C.) 2006, Abst 512-P.