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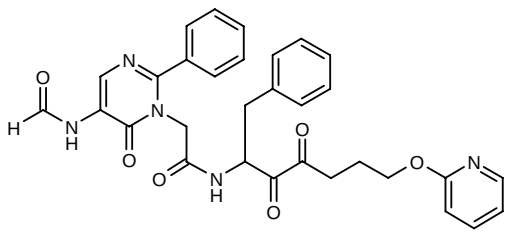
# NK-3201

*Treatment of Restenosis*
*Treatment of Myocardial Infarction*
*Chimase Inhibitor*

*N*-[1-Benzyl-2,3-dioxo-6-(2-pyridyloxy)hexyl]-2-[5-(formylamino)-6-oxo-2-phenyl-1,6-dihydropyrimidin-1-yl]acetamide



$C_{31}H_{29}N_5O_6$
Mol wt: 567.5991
CAS: 204460-24-2
EN: 267345

**Abstract**

Evidence is emerging that chymase may have a major role in the local conversion of angiotensin I to angiotensin II in injury. Oral administration of the chymase inhibitor NK-3201 to dogs reduced neointimal thickening in grafted veins and in balloon injury to the carotid artery. Oral NK-3201 also reduced mortality in hamsters following coronary artery ligation. These results suggest that NK-3201 may be useful in the treatment of restenosis and myocardial infarction.

## Synthesis

Protection of the hydroxy amino ester (I) by heating with 2,2-dimethoxypropane (II) in the presence of *p*-toluenesulfonic acid provides the oxazolidine (III), which is converted into the aldehyde (V) by reduction with $LiAlH_4$ in THF to give the alcohol (IV), followed by oxidation with NaOCl catalyzed by 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and NaBr. Addition of 3-(benzyloxy)propylmagnesium bromide (VI) to aldehyde (V) furnishes carbinol (VII), which is further protected as the silyl ether (VIII) by treatment with *tert*-butyldimethylsilyl chloride and imidazole. Hydrogenolysis of the *O*-benzyl protecting group of (VIII) with $H_2$ over Pd results in the primary alcohol (IX), which is treated with *N*-bromosuccinimide and triphenylphosphine to yield the bromide derivative (X). Alkylation of 2-hydroxypyridine (XI) with bromide (X) produces a mixture of the *N*-alkylated pyridone (XII) and the pyridyl ether (XIII), which are separated by column chromatography. The required minor regioisomer, ether (XIII), is desilylated with tetrabutylammonium fluoride, and the resultant alcohol (XIV) is oxidized under Swern conditions to give ketone (XV). The acidic hydrolysis of the cyclic aminal function of compound (XV) affords the amino alcohol (XVI), which is then coupled with the pyrimidinylacetic acid derivative (XVII) to yield amide (XVIII). Esterification of the free hydroxy group of (XVIII) with acetic anhydride provides ester (XIX), which by acidic Boc group cleavage with HCl in dioxan produces amine (XX). Treatment of amine (XX) with *in situ*-generated formic acetic anhydride affords formamide (XXI), which is submitted to selective hydrolysis of the acetate ester by treatment with $K_2CO_3$ in MeOH to yield the keto alcohol (XXII). Finally, compound (XXII) is oxidized employing DMSO in the presence of EDC and pyridinium trifluoroacetate (1). Scheme 1.

## Introduction

Recent attention has focused on chymase as an alternative pathway to angiotensin-converting enzyme (ACE) in the production of angiotensin II, especially as the chymase pathway seems to be activated in injury. Chymase is a chymotrypsin-like serine protease contained in the secretory granules of mast cells. Upon release, chymase binds to the extracellular matrix and continues to function for several weeks. In addition to the synthesis of angiotensin II, chymase is involved in transforming growth factor-β activation (2) and cleaves type I procollagen to produce collagen (3).

Recent evidence with isolated human tissues suggests that chymase may have a major role in the local conversion of angiotensin I to II in normal hearts (4), mammary arteries (5), coronary arteries (6) and resistance arteries from patients with heart failure or coronary

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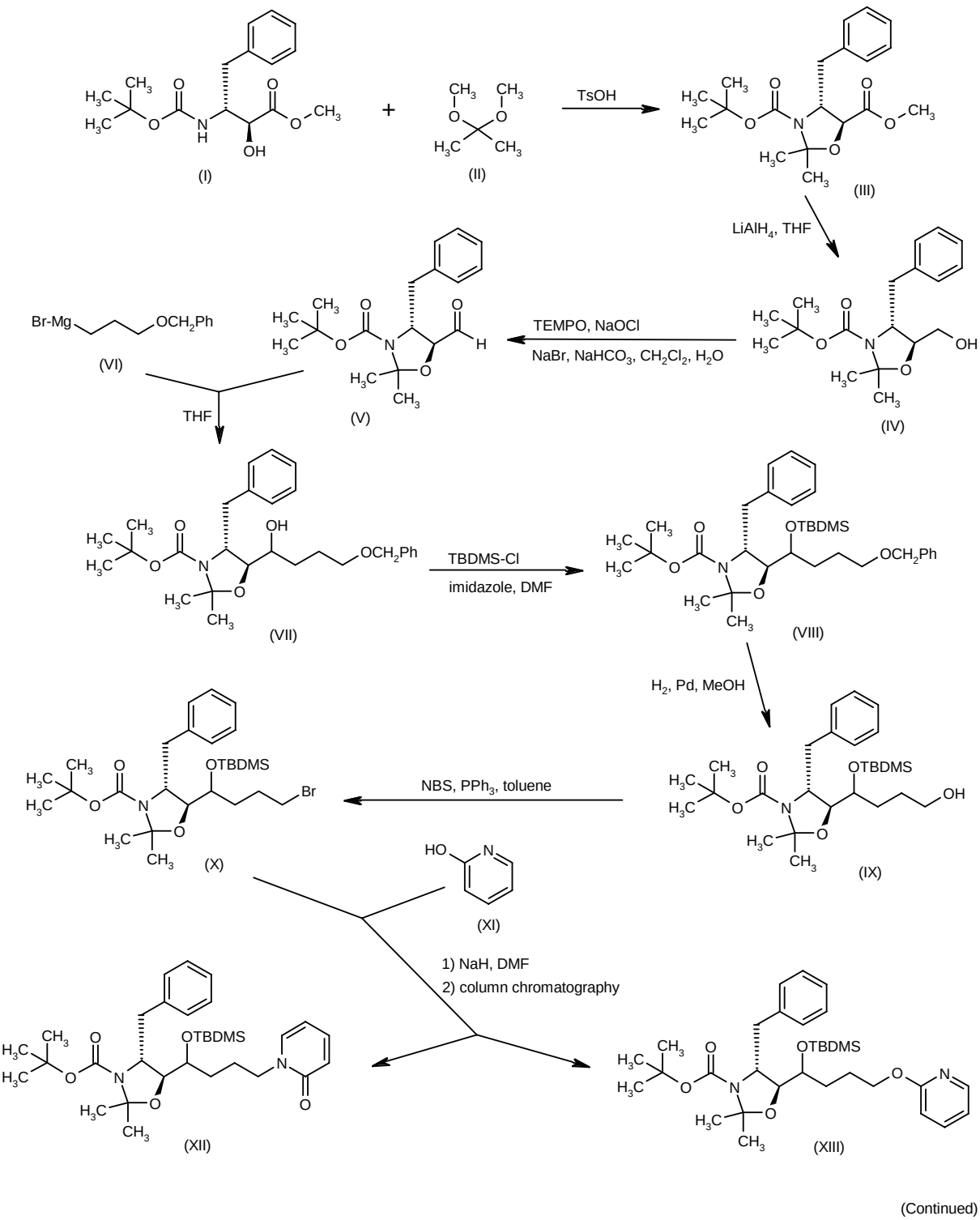
**Scheme 1: Synthesis of NK-3201**

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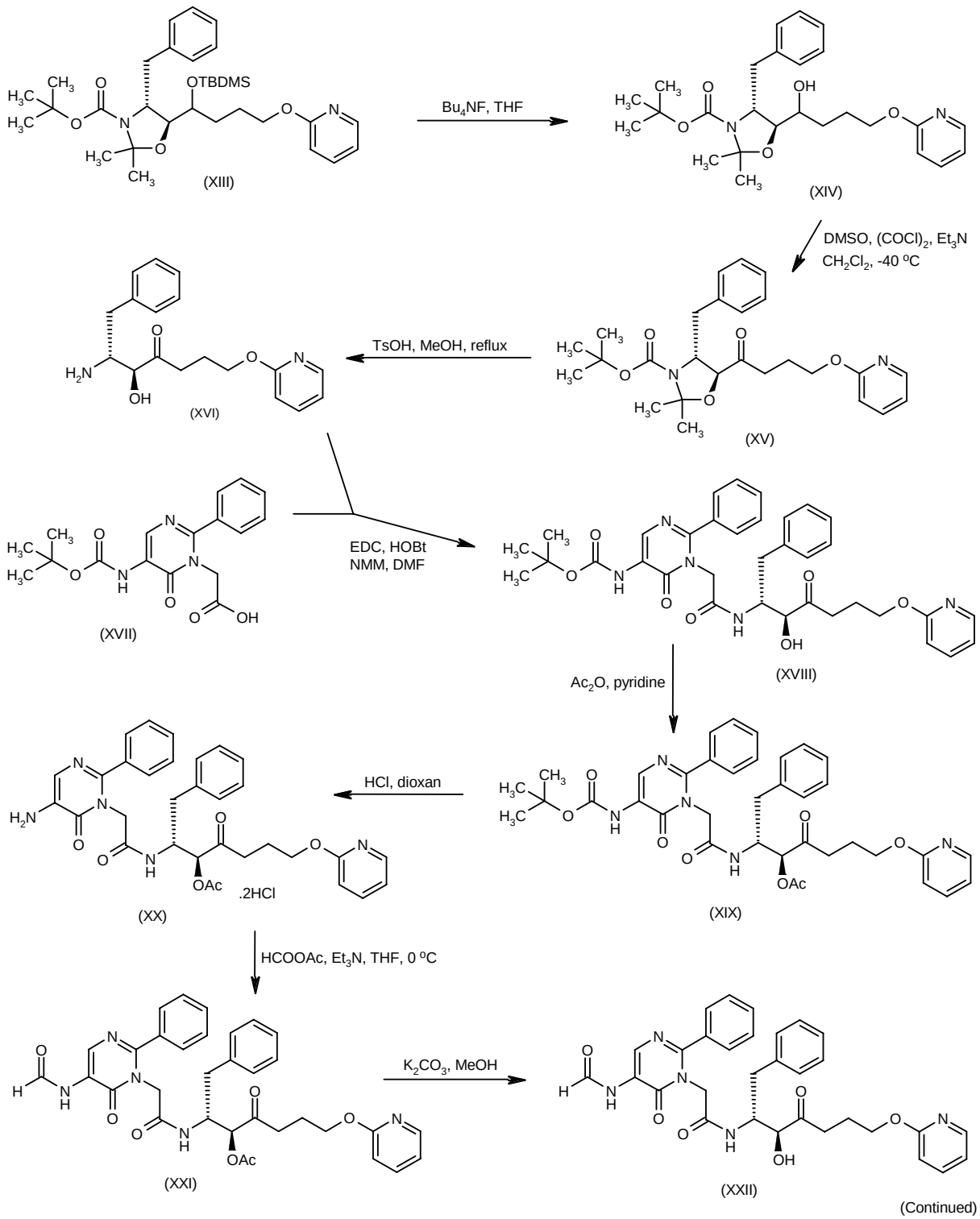
**Scheme 1 (Cont.): Synthesis of NK-3102**

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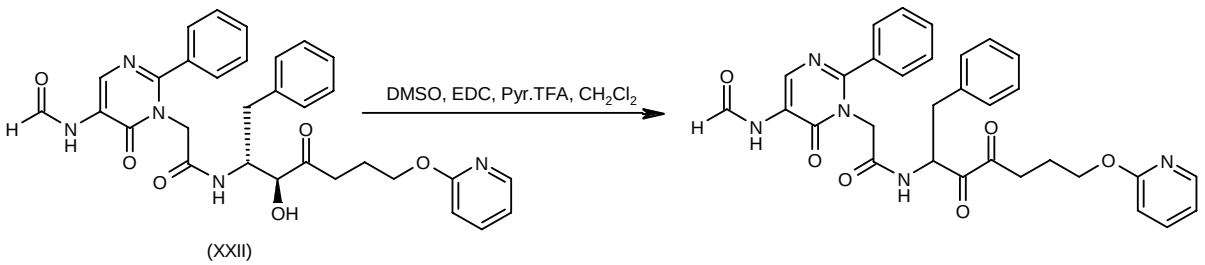
**Scheme 1 (Cont.): Synthesis of NK-3201**




(XXII)

heart disease (7). However, this may be an artificial finding in that the isolation of tissues may be associated with injury leading to the degranulation of mast cells. *In vivo* studies in human dorsal veins of patients with coronary heart disease showed that the ACE inhibitor captopril abolished responses to angiotensin I, which suggests that ACE is the enzyme predominantly being used in the formation of angiotensin II. However, when [Pro$^{11}$,D-Ala$^{12}$]-angiotensin I was used as the substrate, contractions to formed angiotensin II were observed despite the substrate being ACE-resistant. Furthermore, the contractions to angiotensin II from [Pro$^{11}$,D-Ala$^{12}$]-angiotensin I were resistant to captopril. As chymase does catalyze the conversion of [Pro$^{11}$,D-Ala$^{12}$]-angiotensin I to angiotensin II, this suggests that chymase must be active in the human dorsal vein *in vivo* (8).

Chymase activity may be increased in atherosclerosis and restenosis. In the internal thoracic arteries of patients with hypercholesterolemia, most of the angiotensin II forming activity is due to chymase (9). As angiotensin II stimulates vascular proliferation, ACE inhibitors were tested and shown to be of benefit in rat models of restenosis but not in clinical trials. This is possibly because ACE is the only angiotensin II forming enzyme in rats, whereas chymase is present in dogs and humans and may be the major enzyme for the formation of angiotensin II in injury.

## Pharmacological Actions

NK-3201 has been shown to be an orally active inhibitor of chymase. It inhibited recombinant human chymase and dog and hamster chymases isolated from vascular tissue with $IC_{50}$ values of 2.5, 1.2 and 28 nM, respectively. NK-3201 had no effect on other types of serine proteases, and at 100 μM or less had no effect on the ability of rabbit ACE to convert angiotensin I to angiotensin II. Carotid arteries isolated from dogs contracted to angiotensin I in the presence of the ACE inhibitor lisinopril. These contractions were inhibited by NK-3201 with an $IC_{50}$ of 320 nM (10).

In a dog model of artery bypass grafting, dogs were treated with NK-3201 (5 mg/kg/day p.o.) for 5 days before grafting up to 28 days after the operation, at which time the grafted veins were removed. During oral treatment with NK-3201, the dogs appeared healthy and the concentration of NK-3201 in the blood was about 10 μM at 24 h after oral administration. In untreated dogs, chymase activity increased 20-fold whereas ACE activity only increased 1.6-fold compared to normal veins. NK-3201 suppressed the increased chymase activity in grafted veins by 64%. NK-3201 also prevented proliferation in the grafted veins with the ratio of intimal to medial area in the grafted veins being 42% less than in untreated grafted veins (10).

NK-3201 was also beneficial in a dog model of balloon angioplasty. In this model, a balloon catheter was inserted via the right thyroid artery into the right common carotid artery. The balloon was filled with water to distend the artery, injuring the artery internally. The left common carotid artery was kept intact and used as the control artery. Some dogs were untreated, whereas others were treated with NK-3201 (1 mg/kg/day) starting 5 days before injury and continuing until 28 days after the injury. The concentrations of NK-3201 in plasma, heart and aorta were approximately 470, 195 and 78 nM, respectively, 8 h after administration. NK-3201 had no effect on mean blood pressure of the dogs, or plasma levels of renin, ACE and angiotensin II. When the arteries were removed, there was a 2-fold increase in the chymase activities in the injured arteries, but no significant difference in ACE activity. NK-3201 reduced the increased chymase activity by 66%. Neointimal thickening was observed in the injured arteries, and NK-3201 reduced this by 57% (11). As there were no increase in the circulating levels of angiotensin II or blood pressure, this suggests that the activity of chymase and generation of angiotensin II is localized to the area of injury.

Preliminary data suggests that NK-3201 may be useful in the treatment of myocardial infarction. Hamsters have the same type of chymase as humans. Coronary ligation in the hamster resulted in decreases in heart rate, blood pressure and cardiac pressure development and a

mortality rate of 61% at 14 days. Although oral treatment with NK-3201 (30 mg/kg) 3 days before ligation had no effect on heart rate and blood pressure, it did improve heart pressure development and reduce mortality to 17% (12).

NK-3201 is in preclinical development for the treatment of atherosclerosis and restenosis.

## Source

Nippon Kayaku Co., Ltd. (JP); codeveloped with the Department of Pharmacology, Osaka Medical College, Osaka (JP).

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