Pergamon

Tetrahedron Letters, Vol. 36, No. 37, pp. 6721-6724, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01361-X

Total Synthesis of Nagstatin, an *N*-Acetyl-β-D-glucosaminidase Inhibitor

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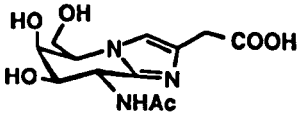
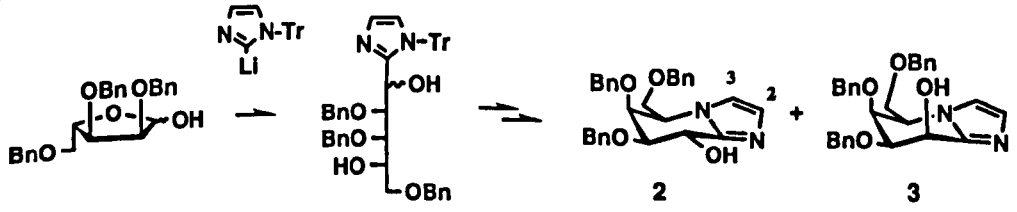
Abstract : The first enantiospecific total synthesis of nagstatin has been accomplished through the regioselective introduction of an allyl group on the imidazole ring of the de-branched nagstatin precursor followed by conversion into the carboxymethyl group, and the stereospecific introduction of the acetamido group.

In recent years, much attention has focused on the synthesis and development of glycosidase inhibitors.[1,2] Nagstatin was discovered in the fermentation broth of *Streptomyces amakusaensis* as a strong and novel inhibitor of *N*-acetyl-β-D-glucosaminidase (IC_{50} 0.0012 μg/ml).[3,4] In several diseases such as diabetes mellitus, leukemia and cancer, *N*-acetyl-β-D-glucosaminidase activity in serum has been reported to increase.[3] Therefore, the chemical and biochemical studies on nagstatin may enable us to understand the processes of these intractable diseases. Together with its noteworthy biological activity, the unique structure of **1** made nagstatin a worthwhile target for synthesis. We have synthesized de-branched nagstatins[5] from ribo- and xylofuranoses through the inter- and intramolecular nucleophilic reactions with the imidazole moieties, and elucidated the structure-activity relationships as well as the absolute structure of **1**.[6]



1 : Nagstatin

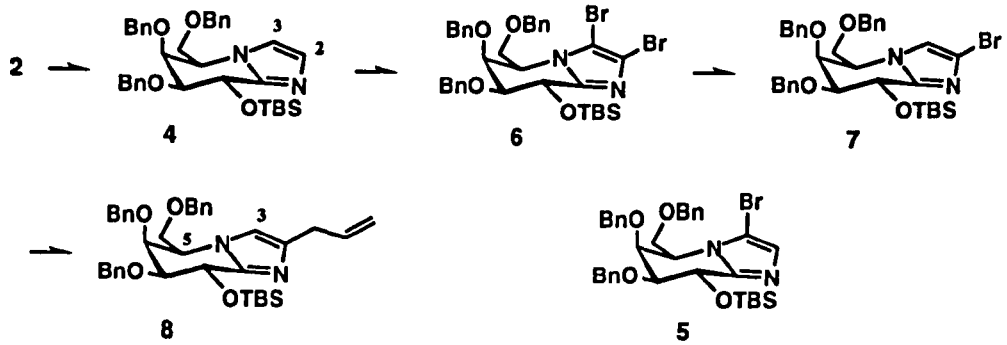
Herein, we report the first enantiospecific total synthesis of nagstatin (**1**). A rational starting point for the synthesis is the imidazole-having isomers **2** and **3**, which have served as key intermediates in our syntheses of de-branched nagstatins.[5]



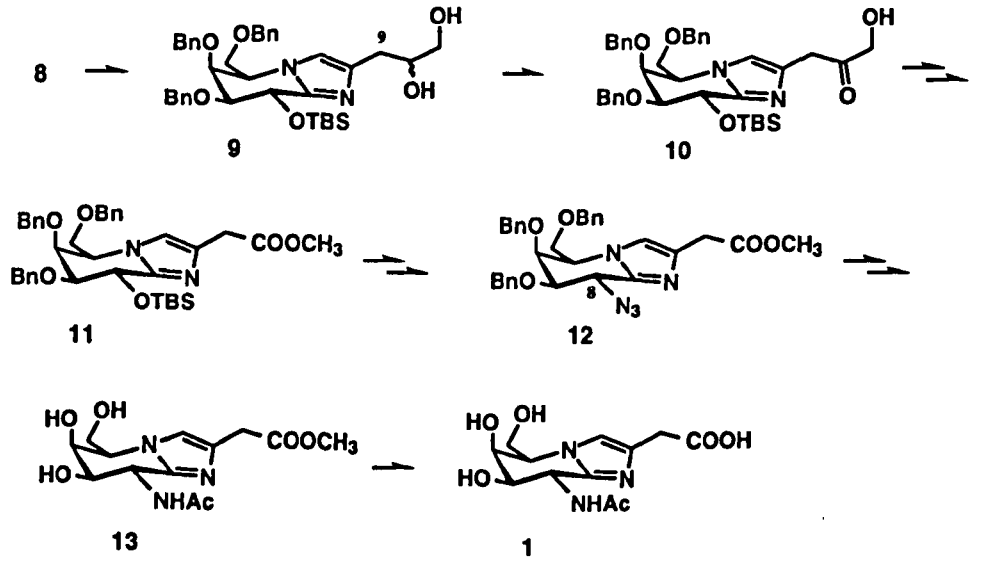
2 **3**

The regioselective introduction of an allyl group on their C-2 positions[7] was investigated under a variety of conditions. The C-2 position is generally known to be less reactive than the C-3.[8] In fact, selective

bromination of **4**, which was quantitatively prepared by silylation (TBSOTf, 2,6-lutidine, CH_2Cl_2, -10°C, 30 min) of **2**, gave the undesired C-3 bromo compound **5**[9]. Accordingly, **4** was fully brominated (92%, 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one, $NaHCO_3$, CH_2Cl_2) to the dibromo compound **6**, the selective debromination of which was assayed.[10] The best result was realized by regioselective lithiation (t-BuLi, THF, -78°C) followed by quenching with H_2O to give the desired monobromo compound **7**[9] [89%, syrup, $[\alpha]_D$ +50°(*c* 1.3, $CHCl_3$)]. The structure was confirmed by the ^{1}H-NMR NOE studies of the corresponding allyl derivative **8**[9][syrup, $[\alpha]_D$ +46°(*c* 1.3, $CHCl_3$)], which was obtained in 88% yield from the organocopper derivative of **7** (n-BuLi, CuI, THF, -78°C, 30 min) and allyl bromide.[8] On irradiating at H-3 (δ 6.85), the NOE enhancements of CH_2-5 (δ 3.76: 1.7%) and H-5 (δ 4.45: 3.5%) signals were clearly detected to support the C-2 substituted structure **8**.

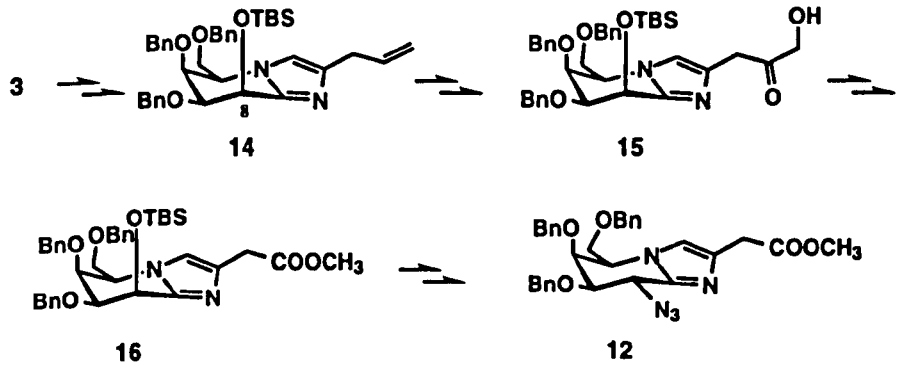


Dihydroxylation (OsO_4, NMO, THF-H_2O, 4 h) of **8** afforded **9** (97%), which was oxidized by using Fetizon's modified conditions[11] (Ag_2CO_3, PhH, reflux, 12 h) to give the keto-alcohol **10**[9] [45%, syrup, $[\alpha]_D$ +50°(*c* 1.9, $CHCl_3$)] with recovery of **9** (40%). Periodate oxidation ($NaIO_4$, MeOH-H_2O, 1 h) of **10** followed by esterification ($TMSCHN_2$, THF-MeOH, 10 min) provided the methyl ester **11**[9] [63%, syrup,



$[\alpha]_D$ +51°(*c* 1.1, $CHCl_3$)]. Direct ozonolysis of **8**, or periodate oxidation of **9** caused concomitant oxidation at C-9 position.

Conversion of **11** to the azido compound **12**[9] [63%, syrup,$[\alpha]_D$ +41°(*c* 1.9, $CHCl_3$)] was carried out by de-*O*-silylation (TBAF, THF, 1 h) followed by treatment with HN_3, n-Bu_3P and DEAD (THF-PhMe, 30 min).[5,6] Expected retention of the configuration was observed at the C-8 position as previously reported by us.[4] Hydrogenolysis (H_2, 10% Pd-C, AcOH, 15 h) of **12** followed by successive *N*-acetylation (Ac_2O, MeOH, 2 h) to yield **13**[9] [55%, mp(dec.)197~199°C(MeOH),$[\alpha]_D$ +88°(*c* 0.3, H_2O)] and saponification (NaOH, H_2O, 5°C, 30 min) provided nagstatin [1·HCl[9]: 84%, mp(dec.)~160°C(amorphous solid),$[\alpha]_D$ +70°(*c* 0.5, H_2O)], which was identical with the natural product[12] in all respects including glycosidase inhibiting activities.



In a similar manner, but with inversion of the configuration,[5] the other C-8 *axial* isomer **3** was converted into the aforesaid azido compound **12** in a ten-step sequence through the allyl derivative **14**[9][syrup, $[\alpha]_D$ -8.0°(*c* 1.1, $CHCl_3$)], the keto alcohol **15**[9][syrup, $[\alpha]_D$ +24°(*c* 1.5, $CHCl_3$)] and the ester **16**[9] [syrup, $[\alpha]_D$ -6.4°(*c* 1.7, $CHCl_3$)] . Thus, de-*O*-silylation of **16** afforded the alcohol, which was submitted to Mitsunobu's S_N2 conditions using HN_3 (n-Bu_3P, DEAD, THF-PhMe, 30 min) to give **12** in 87% yield.[5]

Now, the completion of the synthesis confirmed the absolute structure of nagstatin (**1**).

Acknowledgment: We are grateful to Shikoku Chemicals Co., Yamanouchi Pharmaceutical Co., Ltd. and the Institute of Microbial Chemistry for the generous support of our program.

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7. The carbon-numbering protocol is conveniently according to the first paper of the structural elucidation of nagstatin: See Ref. 4.
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9. All compounds were purified by silica-gel column chromatography and/or recrystallization, and were fully characterized by spectroscopic means. Optical rotations were measured using a 0.5 dm tube at 22°C. Significant ^{1}H-NMR spectral data (270 and 400 MHz, $CDCl_3$, δ; TMS=0, unless otherwise noted) are the following.
1·HCl (400MHz, D_2O): δ 2.14(3H, s), 3.86(2H, br s), 4.18(2H, m), 4.29(1H, dd, *J*=10 & 2Hz), 4.52(1H, m), 4.55(1H, m), 5.19(1H, d, *J*=10Hz), 7.65(1H, s).
5 (270MHz): δ 3.61(1H, dd, *J*=10.5 & 3.5Hz), 3.88(1H, dd, *J*=3.5 & 2Hz), 4.36(1H, dd, *J*=6 & 2Hz), 4.43(1H, dd, *J*=10.5 & 4Hz), 4.76(1H, d, *J*=3.5Hz), 7.03(1H, s).
7 (270MHz): δ 3.75(1H, dd, *J*=10 & 8.5Hz), 3.85(1H, dd, *J*=4 & 2Hz), 4.03(1H, dd, *J*=10 & 2Hz), 4.34(1H, dd, *J*=6 & 2Hz), 4.43(1H, ddd, *J*=8.5, 6 & 2Hz), 4.84(1H, d, *J*=4Hz), 7.09(1H, s).
8 (270MHz): δ 3.34(2H, m), 3.76(1H, dd, *J*=10 & 8Hz), 3.87(1H, dd, *J*=4 & 2Hz), 4.12(1H, dd, *J*=10 & 2Hz), 4.38(1H, dd, *J*=6 & 2Hz), 4.45(1H, ddd, *J*=8, 6 & 2Hz), 4.92(1H, d, *J*=4Hz), 5.05(1H, m), 5.13(1H, m), 6.01(1H, dddd, *J*=17, 10, 7 & 7Hz), 6.86(1H, s).
10 (270MHz): δ 3.69(1H, d), 3.76(1H, d), 3.77(1H, dd), 3.83(1H, dd), 4.04(1H, dd), 4.32(1H, dd), 4.40(1H, ddd), 4.49(2H, m), 4.85(1H, d), 6.98(1H, s).
11 (270MHz): δ 3.59(1H, d), 3.62(1H, d), 3.68(3H, s), 3.76(1H, dd), 3.86(1H, dd), 4.10(1H, dd), 4.35(1H, dd), 4.45(1H, ddd), 4.88(1H, d), 7.05(1H, s).
12 (270MHz): δ 3.64(2H, m), 3.69(3H, s), 3.7~3.9(3H, m), 4.1~4.3(2H, m), 4.98(1H, d, *J*=7.5Hz), 7.06(1H, s).
13 (270MHz, D_2O): δ 2.10(3H, s), 3.66(2H, m), 3.72(3H, s), 4.04(1H, dd, *J*=11 & 6Hz), 4.1~4.2(2H, m), 4.29(1H, m), 4.46(1H, dd, *J*=2 & 2Hz), 5.03(1H, d, *J*=9.5Hz), 7.23(1H, s).
14 (270MHz): δ 3.32(2H, m), 3.89(1H, dd, *J*=10 & 8Hz), 3.9~4.0(2H, m), 4.04(1H, dd, *J*=10 & 2Hz), 4.28(1H, m), 4.88(1H, d, *J*=4Hz), 5.03(1H, m), 5.13(1H, m), 6.03(1H, m), 6.85(1H, s).
15 (270MHz): δ 3.65(1H, d), 3.82(1H, d), 3.86(1H, dd), 3.94(1H, dd), 4.00(1H, dd), 4.08(1H, dd), 4.23(2H, m), 4.36(1H, m), 4.77(1H, d), 6.99(1H, s).
16 (270MHz): δ 3.61(2H, m), 3.70(3H, s), 3.90(1H, dd), 3.9~4.0(2H, m), 4.05(1H, dd), 4.33(1H, m), 4.86(1H, d), 7.07(1H, s).
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12. The authentic sample of natural nagstatin was kindly provided by Dr. T. Aoyagi.[3)]

(*Received in Japan* 9 *June* 1995; *revised* 17 *July* 1995; *accepted* 20 *July* 1995)