Total Synthesis of (+)-Blasticidin S

Yoshiyasu Ichikawa,* Keiko Hirata, Masayoshi Ohbayashi, and Minoru Isobe[a]

Dedicated with respect and appreciation to Professor Ōtake on the occasion of his 77th birthday

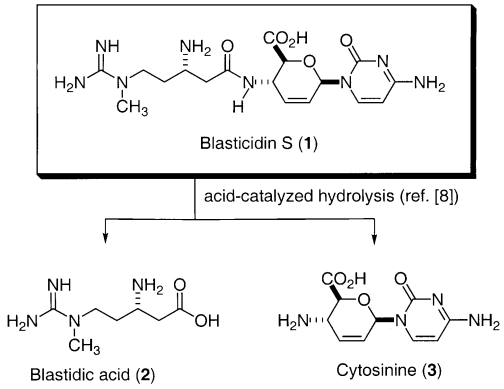
Abstract: The first total synthesis of the peptidyl nucleoside antibiotic, blasticidin S (**1**), has been achieved by the coupling reaction of cytosinine (**3**) and blastidic acid (**2**). A key step in the synthesis of cytosinine (**3**) is the sigmatropic rearrangement of allyl cyanate **24**; this reaction provided efficient and stereoselective access to 2,3-dideoxy-4-amino-D-hex-2-enopyranose (**26a**). Further elaboration of **26a** gave methyl hex-2-enopyranouronate **29**, and cytosine N-glycosylation of **31** using the Vorbrüggen conditions for the silyl Hilbert–Johnson reaction furnished the differentially protected cytosinine (**32**) in 11 steps from 2-acetoxy-D-glucal (**14**) (4.0% overall yield). Synthesis of the Boc-protected blastidic acid **47** in nine steps starting from chiral carboxylic acid **35** (23% overall yield) utilized Weinreb's protocol for the preparation of benzyl amide **38** and Fukuyama's protocol for the synthesis of the secondary amine **40**. Assembly of the protected cytosinine (**32**) and blastidic acid (**47**) by the BOP method in the presence of HOBt, and finally elaboration to **1** by deprotection of the fully protected **54** established the total synthesis of blasticidin S (**1**).

Keywords: amino acids · blasticidin S · natural products · rearrangement · total synthesis

Introduction

Blasticidin S (**1**) is a representative peptidyl-nucleoside antibiotic,[1] which was first isolated from *Streptomyces griseochromogenes* in 1958 by Yonehara and co-workers.[2] This antibiotic was once commercialized as a fungicide against the virulent fungus, *Piricularia oryzae*, which was the cause of the serious rice blast disease in Asia. Its biological activity results from specific inhibition of the protein biosynthesis by interfering with the peptide bond formation in the ribosomal machinery.[3] Biosynthetic studies by Gould have advanced to the molecular level, whereby biosynthetic gene clusters have been cloned and expressed.[4] Yamaguchi found two blasticidin S resistance genes which code blasticidin S deaminase: *bsr* from *Bacillus cereus*[5] and BSD from *Aspergillus terreus*[6]. Both genes are now widely used for genetic engineering experiments to select both prokaryotic and eukaryotic cells that express the blasticidin S resistance gene. The renaissance of blasticidin S is now flourishing in the research area of molecular biology.[7]

The structure and absolute configuration of **1** have been elucidated by chemical degradation and spectroscopic studies by Ōtake and co-workers,[8] and has also been confirmed by X-ray analysis (Scheme 1).[9] Controlled acid hydrolysis of **1** allowed the isolation of two components, blastidic acid (**2**) and cytosinine (**3**) as their hydrochloride salts (Scheme 1). Blastidic acid (**2**) is an unusual β-amino acid counterpart of arginine containing a modified N-methyl guanidine group.[10] Such a β-amino acid motif is also manifested in cytosinine (**3**). The highly functionalized structure in **3** is characterized by a unique hexopyranosyl nucleoside that contains a 2,3-unsaturated-4-amino pyranose attached to cytosine.[11] The functional group richness found in blasticidin S poses synthetic challenges.



Scheme 1. Structures of blasticidin S, blastidic acid, and cytosinine.

Although the pioneering work of Kondo and Goto[12] detailed the first synthesis of cytosinine in 1972, and two re-

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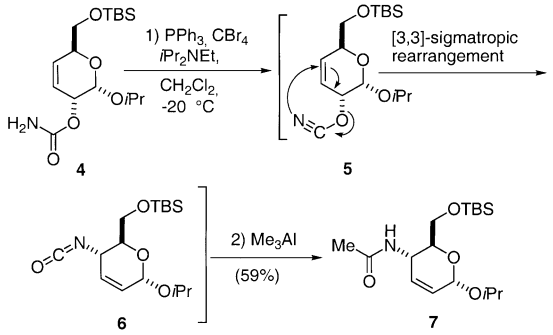
ports for the syntheses of blastidic acid appeared in 2001,[13] the total synthesis of **1** has not yet been reported. In this manuscript, we discuss the evolution of our strategy for the synthesis of cytosinine in full detail, and elaborate the first total synthesis of blasticidin S.

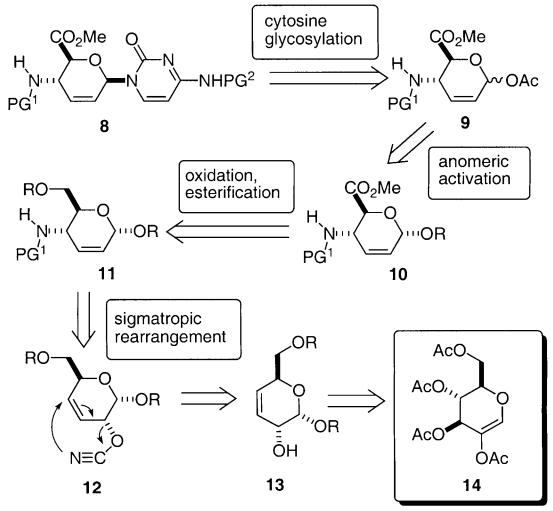
Synthetic analysis of cytosinine (3): We envisioned that the suitably protected cytosinine and blastidic acid intermediates could be coupled at a late stage in the synthesis. Accordingly, our initial efforts were devoted to the synthesis of these two components. Several years ago, we reported a synthetic method for the preparation of the unsaturated amino sugar, 2,3-dideoxy-4-amino-D-hex-2-enopyranose (**7**),[14] by an allyl cyanate-to-isocyanate rearrangement[15] (Scheme 2).



Scheme 2. Synthesis of 2,3-dideoxy-4-amino-D-hex-2-enopyranose by allyl cyanate-to-isocyanate rearrangement.

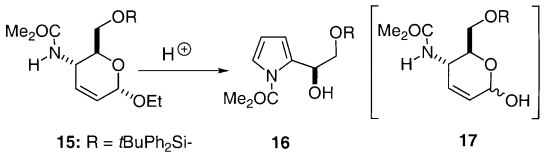
Dehydration of allyl carbamate **4** with triphenylphosphine (PPh_3), carbon tetrabromide (CBr_4), and diisopropylethylamine (iPr_2NEt) under modified Appel's condition[16] provided allyl cyanate **5**, which underwent [3,3]-sigmatropic rearrangement to afford allyl isocyanate **6**. Subsequent treatment of **6** with trimethylaluminum afforded unsaturated aminopyranose **7** in 59% yield.

In this study we reasoned that our method would provide a convenient approach to the central core of cytosinine, and this consideration led to the retrosynthetic strategy outlined in Scheme 3. Synthesis of the fully protected cytosinine **8** was envisioned to arise from anomeric activation of **10** followed by introduction of the cytosine moiety. Since the acyl protecting group on the cytosine N^4-nitrogen (PG^2 in **8**) is not sufficiently robust to carry out a sequence of manipulations,[17] the ester functionality was to be installed prior to the cytosine N-glycosylation. We further reasoned that unsaturated aminopyranoside **11** could be elaborated by [3,3]-sigmatropic rearrangement of allyl cyanate **12**, which itself would be derived from 2-acetoxy-D-glucal (**14**). Based on this synthetic analysis, we launched a synthetic venture toward cytosinine (**3**).



Scheme 3. Strategy for the synthesis of cytosinine.

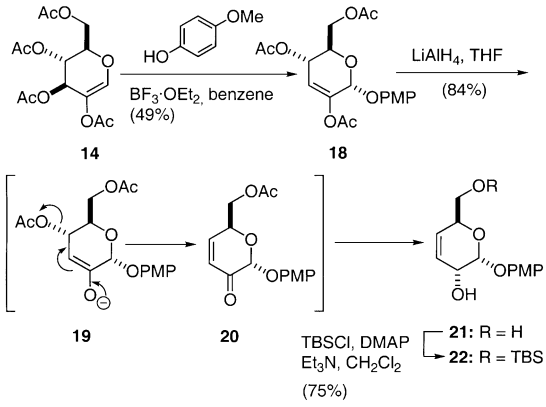
Synthesis of the fully protected cytosinine 32: In our preliminary investigations, we found that acid-catalyzed hydrolysis of a 2,3-unsaturated 4-aminopynanoside such as **15** was problematic (Scheme 4). Numerous attempts to obtain lactol



Scheme 4. Acid-catalyzed hydrolysis of 2,3-unsaturated-4-aminopynanoside.

17 by acid-catalyzed hydrolysis of **15** were unsuccessful, and only 2-substituted pyrrole **16** was isolated.

To circumvent this problem, we selected *p*-methoxyphenyl glycoside **18** as a starting material (Scheme 5), because such

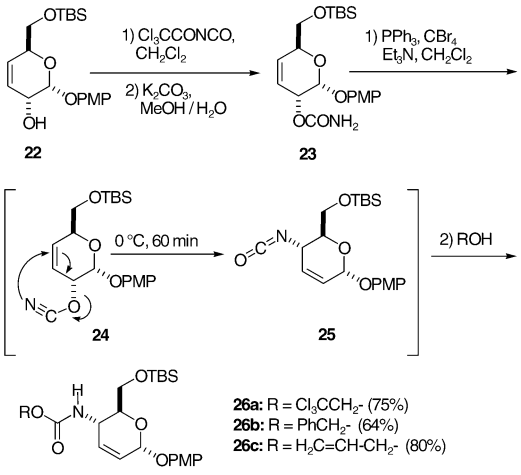


Scheme 5. Synthesis of hex-3-enopyranoside from 2-acetoxy glucal.

a glycoside could be oxidatively hydrolyzed under mild conditions.[18] As a result, synthesis of cytosinine began with a Ferrier-type glycosylation of 2-acetoxy-tri-*O*-acetyl-D-glucal (**14**) using *p*-methoxyphenol and $BF_3{\cdot}OEt_2$ as the catalyst. The resulting *p*-methoxyphenyl glycoside **18** was obtained as a crystalline solid in 49% yield.[19] Treatment of **18** with $LiAlH_4$ initiated a cascade reaction, which involves enol acetate cleavage, β-elimination of the resulting enolate **19**, and nucleophilic hydride addition to **20** from the sterically less-hindered β-face[20] to furnish diol **21** exclusively in 84% yield. The primary alcohol of **21** was then selectively pro-

tected with *tert*-butyldimethylsilyl (TBS) chloride in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) and Et_3N to afford TBS ether **22** in 75% yield.[21]

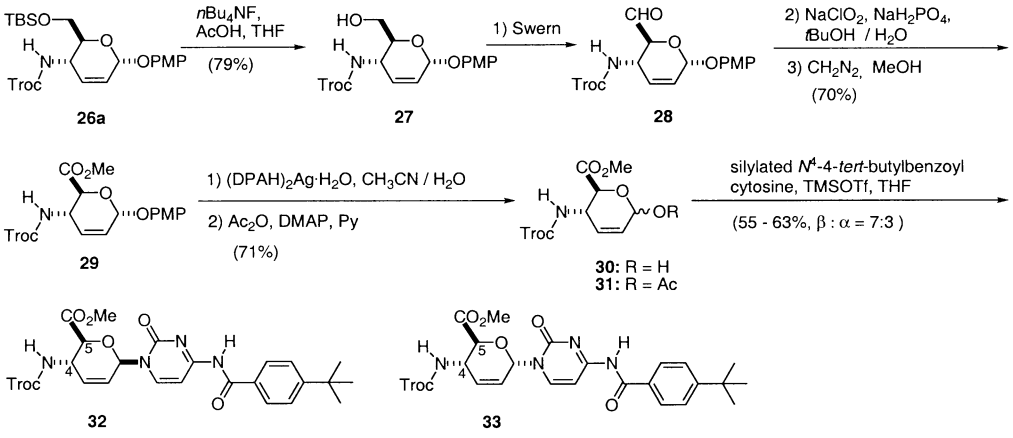
With the hex-3-enopyranoside **22** in hand, the next goal was to synthesize the 4-amino-hex-2-enopyranose using an allyl cyanate-to-isocyanate rearrangement (Scheme 6).



Scheme 6. Allyl cyanate-to-isocyanate rearrangement for the construction of the 4-amino-hex-2-enopyranose component in cytosinine.

Treatment of **22** with trichloroacetyl isocyanate followed by hydrolysis with potassium carbonate (K_2CO_3) in aqueous methanol provided allyl carbamate **23**. Dehydration of **23** (PPh_3, CBr_4, Et_3N) gave allyl cyanate **24**, which then underwent [3,3]-bond reorganization at 0°C (60 min) to afford allyl isocyanate **25**. Since isolation of **25** using aqueous workup would result in lower yields, isocyanate **25** was treated in situ with 2,2,2-trichloroethanol[22] to give trichloroethoxy (Troc) carbamate **26a** in 75% overall yield from **22** after chromatographic purification. Carbamates **26b** (Cbz) and **26c** (Alloc) were also prepared in a similar manner in 64 and 80% yields, respectively, when either benzyl alcohol or allyl alcohol was employed in the reaction with **25**. After considerable experimentation, we elected to use Troc carbamate **26a** rather than **26b** or **26c** in subsequent reactions, because we experienced better results for the cytosine N-glycosylation step with **26a**, and deprotection of the carbamate could be carried out under milder conditions.[23]

With a viable route to the unsaturated amino carbohydrate **26a** having been established, we next focused on the transformation of **26a** into the corresponding methyl hex-2-enopyranouronate and its subsequent cytosine N-glycosylation (Scheme 7). The TBS group in **26a** was removed with tetrabutylammonium fluoride (*n*Bu_4NF) buffered with acetic acid (AcOH) in THF to provide **27** in 79% yield.[24] Swern oxidation (oxalyl chloride, DMSO, Et_3N, CH_2Cl_2) of **27** gave the rather labile aldehyde **28**, which was immediately subjected to sodium chlorite oxidation ($NaClO_2$, NaH_2PO_4, 2-methyl-2-butene). Subsequent treatment of the resultant carboxylic acid with diazomethane in methanol furnished methyl ester **29** as a white powdery solid in 70% overall yield for the three steps. Hydrolysis of *p*-methoxyphenyl glycoside **29** was carried out with silver(II) bis(hydrogen dipicolinate) in aqueous acetonitrile.[25] Unfortunately, we found that concentration of the CH_2Cl_2 extracts containing the fragile lactol **30** often resulted in a tarry oil from which pyrrole-like products could be identified by ^{1}H NMR spectroscopy. To circumvent this problem, the CH_2Cl_2 extracts were immediately treated with acetic anhydride, DMAP, and pyridine; this protocol successfully provided acetyl glycoside **31** as an anomeric mixture (α:β 3:1) in 71% yield.[26] Since we had succeeded in anomeric activation, we subsequently attempted the cytosine N-glycosylation of **31**. After screening several silylated cytosine derivatives and Lewis acids, the following points were observed: 1) we selected the 4-*tert*-butylbenzoyl group to protect the cytosine N^4-nitrogen,[27] because products that contain this liphophilic protecting group behave better chromatographically during efforts to separate such anomeric mixtures than the corresponding acetyl and benzoyl counterparts; 2) we found that Lewis acids such as $SnCl_4$ and BF_3·OEt_2 could not be used because only pyrrole-like compounds were detected by ^{1}H NMR analysis of the crude products.[28] Fortunately, the Vorbrüggen method using trimethylsilyl triflate (TMSOTf) was found to be successful;[29] and 3) it was found that selectivity and yield depended on the solvent and amount of cytosine used.[30] β-Selectivity was optimal when the reaction was carried out in

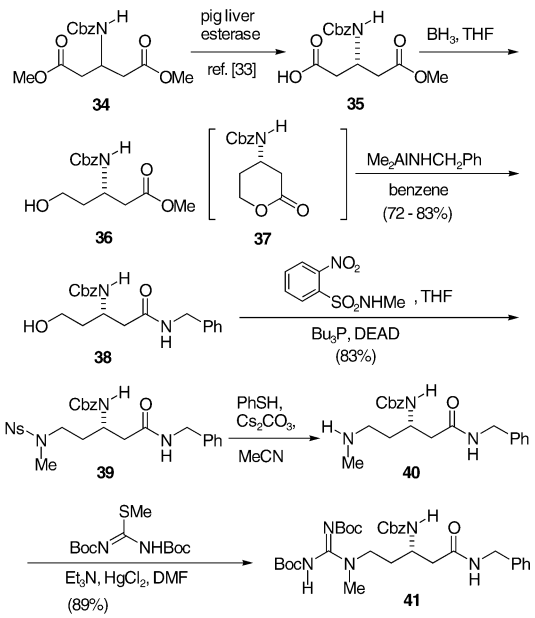


Scheme 7. Final elaboration for the synthesis of the fully protected cytosinine.

THF and excess use of N^4-(4-*tert*-butylbenzoyl)cytosine (ca. 6–8 equiv) resulted in reproducible and better yields.

Reaction of N^4-(4-*tert*-butylbenzoyl)cytosine with *N,O*-bis(trimethylsilyl)acetamide (THF, room temperature, 30 min) gave silylated cytosine, which was subsequently treated with **31** in the presence of TMSOTf at 0 °C for 2 h. After workup, a 7:3 mixture of **32** and **33** was obtained in 55–63 % yield in which the desired β-isomer predominated.[31] Careful separation of the mixture by silica-gel chromatography and repeated recrystallization afforded the fully protected cytosinine **32** as a glassy wax. The stereochemistry of **32** was determined on the basis of ^{1}H NMR coupling constants. The diagnostic coupling constant between H-4 and H-5 for the β-isomer **32** is 9.0 Hz, whereas the α-isomer **33**, in which the cytosine moiety adopts in the pseudo-equatorial position, has a coupling constant of $J_{4,5}=5.5$ Hz.

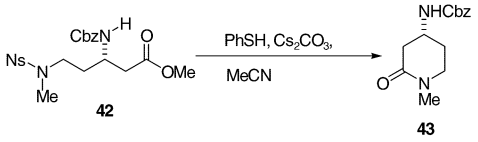
Synthesis of blastidic acid: Retrosynthetic analysis of blastidic acid (**2**) revealed that this β-amino acid possesses a hidden symmetry. Therefore, the synthesis began with the preparation of chiral carboxylic acid **35** by enantioselective hydrolysis of *meso*-diester **34**[32] using pig liver esterase[33] (Scheme 8).



Scheme 8. Synthesis of blastidic acid statrting from *meso*-diester.

Although diborane reduction of carboxylic acid **35** yielded alcohol **36**, sometimes small amounts of lactone **37** were formed during workup. Moreover, preliminary experiments revealed that methyl ester **42** rapidly cyclized to form lactam **43** upon removal of the sulfonyl group (Scheme 9).

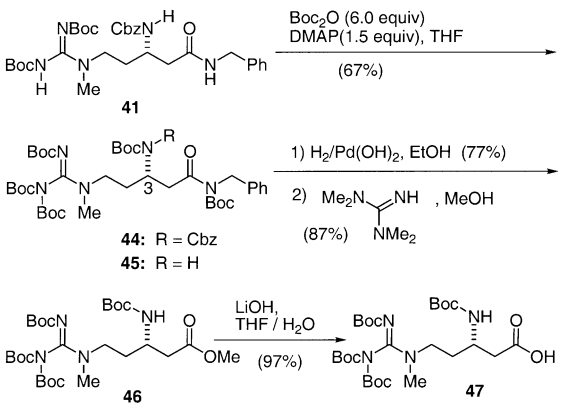
To avoid these problems, methyl ester **36** was transformed into benzyl amide **38**.[34] In practice, the crude product **36** was treated with benzylamine in refluxing benzene. This gave **38** in 74 % yield on a 200 mg scale, while on larger scales the yield decreased to less than 50 %. As a result of this problem another method was briefly investigated and a



Scheme 9. Deprotection of nosyl group leading to a spontaneous cyclization to lactam.

reliable procedure on a gram-scale was realized by applying the Weinreb protocol.[35] Treatment of **36** with dimethylaluminum benzylamide ($PhCH_2NHAlMe_2$) in benzene at room temperature consistently provided **38** in yields of 72–83 %. The requisite N-methyl amino substituent was then introduced by Fukuyama's procedure.[36] Thus, substitution of alcohol **38** with *N*-methyl 2-nitrobenzenesulfonamide under Mitsunobu conditions furnished *o*-nitrobenzenesulfonamide **39** in 83 % yield. Deprotection of the *o*-nitrobenzenesulfonyl group in **39** with thiophenol and cesium carbonate in acetonitrile, followed by treatment of the resultant N-methylamine **40** with *N,N*-di-(*tert*-butoxycarbonyl)-*S*-methylisothiourea and mercuric chloride[37] afforded bis(Boc)-protected N-methyl guanidine **41** in 89 % overall yield for the two steps.

With the requisite functional groups for blastidic acid now in place, we turned to the hydrolysis of the benzyl amide in **41**. This involved a two-step procedure; amide activation followed by hydrolysis[38] (Scheme 10). Initial attempts to selectively introduce the *tert*-butoxycarbonyl (Boc) group onto the nitrogen of the benzylamide in **41** [$(Boc)_2O$, DMAP, THF] failed, and only a mixture of N-Boc imides was isolated. Moreover, NMR analysis of the products was complicated by the existence of rotamers and broadening of the spectra. The following observations for the N-Boc carboxylation of model compounds **48** and **49** suggested a solution for this step (Figure 1).



Scheme 10. Synthesis of the Boc-protected blastidic acid.

Firstly, selective N-Boc carboxylation of the benzyl amide in **48** failed, and a mixture of products was isolated. Secondly, the nitrogen in the bis(Boc)-protected N-methyl guanidine **49** also underwent Boc-carboxylation to furnish tris-(Boc)-protected N-methyl guanidine **50**. Thirdly, the com-

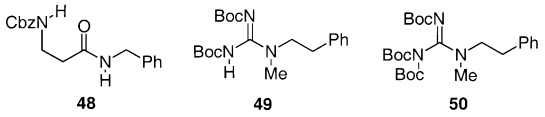
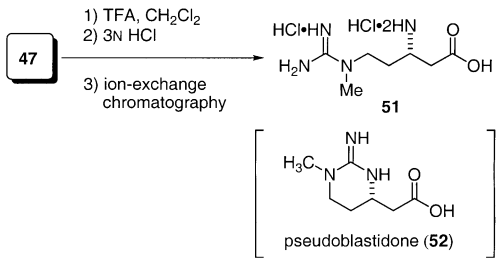


Figure 1. Model compounds for N-Boc carboxylation.

petitive carboxylation of **48** and **49** revealed that bis(Boc)-protected N-methyl guanidine **49** is acylated more rapidly than **48**. Finally, ^{1}H NMR analysis of **50** showed the presence of two rotamers in a 3:1 ratio. Therefore, benzylamide **41** was subjected to exhaustive acylation with di-*tert*-butyl dicarbonate (6.0 equiv) and DMAP (1.5 equiv) in THF to furnish the penta-N-Boc imide **44** in 67 % yield. Unfortunately, saponification of **44** (LiOH, THF, H_2O) gave only complex mixtures; these were presumed to arise from base-catalyzed β-elimination of the imide group at C-3. This undesired elimination could be suppressed by lowering the leaving group ability. Therefore, the Cbz group in **44** was removed by catalytic hydrogenolysis to give the Boc-carbamate **45** in 77 % yield, and methanolysis of **45** with tetramethylguanidine in methanol then cleanly afforded methyl ester **46** in 87 % yield.[39] Finally, hydrolysis of methyl ester **46** with lithium hydroxide in aqueous THF furnished the Boc-protected blastidic acid **47** in 97 % yield.

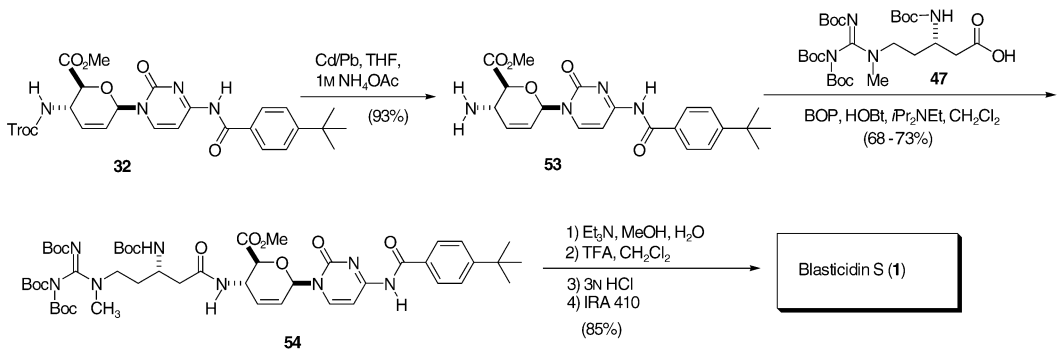
Since NMR analysis of **47** was complicated with extensive broadening, we attempted to prepare blastidic acid dihydrochloride **51** in order to compare it with an authentic sample prepared from natural blasticidin S (Scheme 11). Boc-Protected blastidic acid **47** was thereby treated with trifluoroacetic acid (TFA) in CH_2Cl_2 at room temperature for 2 h. The reaction mixture was then concentrated and dried under vacuum for several hours, and the resultant trifluoroacetate salt was treated with 3 N HCl. To our surprise, ^{1}H NMR analysis indicated that the resultant product was a mixture of blastidic acid and a by-product, which was subsequently identified as pseudoblastidone (**52**).[40] To circumvent the formation of **52**, the crude trifluoroacetate salt of blastidic acid was immediately treated with 3 N HCl and then purified by ion-exchange chromatography. The ^{1}H and ^{13}C NMR spectra for our synthetic **51** were found to be identical to those obtained for a sample that had been prepared from natural blasticidin S.[41]



Scheme 11. Preparation of blastidic acid dihydrochloride.

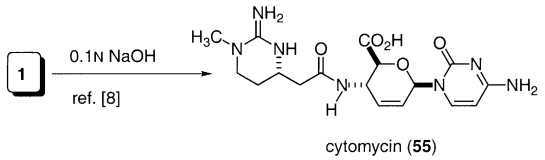
Total synthesis of blasticidin S: With the fully protected cytosinine **32** and blastidic acid **47** in hand, our efforts turned toward coupling these two components (Scheme 12). Initially, we focused on removing the Troc group in **32**; this proved to be more difficult than expected. Attempts to deprotect **32** with zinc (washed with aqueous HCl)[42] and acetic acid in THF afforded non-reproducible results, because deprotection was also accompanied by hydrolysis of the N^4-cytosine *tert*-butylbenzoyl group. We then tried to use zinc that had been activated with TMSCl.[43] Unfortunately, hydrolysis of the sensitive *tert*-butylbenzoyl group as well as mono-dechlorination of the Troc group occurred. After considerable frustration, we were very gratified to find that a cadmium/lead (Cd/Pb) couple reported by Ciufolini[44] was a mild and efficient method for this deprotection. Treatment of **32** with a large excess (40 equiv) of the Cd/Pb couple at room temperature for 30 min afforded the desired amine **53** (93 % based on consumed **32**) together with recovered starting material **32** (13 % recovered) to set the stage for the coupling reaction. After a number of coupling methods for amide bond construction were surveyed, an in situ activation method using the BOP reagent[45] proved to be most effective. In particular, attempts to condense **53** with protected blastidic acid **47** using BOP and diisopropylethylamine (*i*Pr$_2$NEt) gave rise to the desired coupled product **54**, albeit in only 32 % yield. However, addition of 1-hydroxy-benzotriazole (HOBt)[46] improved the yields up to 73 %.

All that remained to complete the total synthesis was to remove the six protecting groups present in **54**. However, we were particularly concerned about the possibility of the blastidic acid moiety undergoing base-catalyzed cyclization



Scheme 12. Total synthesis of blasticidin S.

during the deprotection sequence. Indeed, Ōtake reported that treatment of blasticidin S (**1**) with base afforded cytomycin (**55**)[8] (Scheme 13).



Scheme 13. Base-catalyzed cyclization of blasticidin S to afford cytomycin.

Therefore, deprotection of **54** began with the base-catalyzed hydrolysis of the *tert*-butylbenzoyl protecting group and methyl ester substituent. Treatment of **54** with Et_3N in MeOH promoted cleavage of the labile *tert*-butylbenzoyl group, while addition of water to the reaction mixture resulted in the hydrolysis of the methyl ester group. After the reaction mixture was concentrated, the four Boc protecting groups in the resultant carboxylate were removed by sequential treatment with TFA in CH_2Cl_2 and then 3N HCl. The resulting blasticidin S hydrochloride was purified on Amberlite IRA-410 (OH^- form) to afford blasticidin S (**1**) as the free base in 85% yield.[47] Our synthetic material was found to be identical (^{1}H NMR, ^{13}C NMR, IR, $[\alpha]_D$, TLC) with an authentic sample of natural blasticidin S.[48]

Conclusion

An allyl cyanate-to-isocyanate rearrangement has been successfully employed for the construction of an unsaturated amino sugar moiety in cytosinine. Furthermore, synthesis of the protected cytosinine **32** was achieved in 11 steps starting from 2-acetoxy-tri-*O*-acetyl-D-glucal (**14**) in 4.0% overall yield.[49] The Boc-protected blastidic acid (**47**) was synthesized in nine steps from chiral carboxylic acid **35** (overall yield 23%). The two components **53** and **47** were coupled using the BOP method in the presence of HOBt, and subsequent global deprotection of **54** completed the first total synthesis of blasticidin S (**1**).

Experimental Section

General: Melting points were recorded on a micro melting-point apparatus and are not corrected. Optical rotations are given in units of 10^{-1} deg cm^2 g^{-1}. Infrared spectra are reported in wave number (cm^{-1}). ^{1}H NMR chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) (δ 0.00 in $CDCl_3$), CHD_2OD (δ 3.31 in CD_3OD), *t*BuOH (δ 1.24 in D_2O), or [D$_4$]3-(trimethylsilyl)propionic-2,2,3,3 acid sodium salt (TSP) (δ 0.00 in 1N DCl) as internal standards. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, sext = sextet, br = broadened, m = multiplet), coupling constants (*J*, given in Hz). ^{13}C NMR chemical shifts (δ) are recorded in parts per million (ppm) relative to $CDCl_3$ (δ 77.0), CD_3OD (δ 49.0), or 1,4-dioxane (δ 67.4 in D_2O or 1N DCl) as internal standards. The tri-Boc-N-methyl guanidine derivatives exit as a mixture of rotamers on the NMR time scale. In instances where two rotamers were observed, the carbon signals for the minor rotamers are listed in parentheses. High-resolution mass spectra (HRMS) are reported in *m*/*z*. Elemental analyses were performed by the Analytical Laboratory at the Graduate School of Bioagricultural Sciences, Nagoya University. Reactions were run under an atmosphere of nitrogen if the reactions were sensitive to moisture or oxygen. Dichloromethane was dried over molecular sieves (3 Å), while acetonitrile was stored over molecular sieves (4 Å). Pyridine and triethylamine were stored over anhydrous KOH. All other commercially available reagents were used as received.

Mol scale preparation of 2-acetoxy-D-glucal (14): A 5 L three-necked, round-bottomed flask equipped with a mechanical stirrer and a dropping funnel was charged with acetic anhydride (2.0 L) and perchloric acid ($HClO_4$, 30%, 7.0 mL). D-Glucose (500 g, 2.78 mol) was added to this solution portionwise over 2 h. After stirring at room temperature overnight, the reaction mixture was cooled to −10°C and PBr_3 (1.30 kg, 13.7 mol) was added through a dropping funnel. Mechanically efficient stirring was necessary during the addition because, otherwise, pentaacetyl glucopyranoside began to crystallize upon cooling. Water (133 mL, 5.54 mol) was added dropwise to this solution as the temperature was maintained between −5 to −10°C. The cooling bath was removed, the mixture was allowed to stand for 4 d at room temperature, and was then poured into ice water (ca. 7.0 L). The resultant brown mixture was stirred vigorously with a mechanical stirrer as the product gradually solidified. The crude product was filtered and then washed with water. The resultant brown solid was dissolved in CH_2Cl_2 (ca. 800 mL) and the solution was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated to give a viscous oil. This material was dissolved in diethyl ether and then concentrated, and this procedure was repeated until crystallization began to furnish white crystals (974.8 g, 85%). The product was then used in the next reaction without further purification.

DBU (305 mL, 2.04 mol) was added through a dropping funnel to a solution of α-bromo tetra-*O*-acetyl-glucopyranoside (778 g, 1.89 mol) dissolved in DMF (1.70 L) at −10°C. After stirring at −10°C for 60 min, the resultant brown reaction mixture was poured into ice water (ca. 10 L). Crystallization began with the aid of seeding and vigorous scratching. The crude product was filtered and then dried in air to afford the crude solid (533 g). Recrystallization from a mixture of methanol (500 mL) and water (400 mL) afforded **14** (359 g, 57%) as white crystals.

***p*-Methoxyphenyl 2,4,6-triacetyl-3-deoxy-α-D-*erythro*-hex-2-enopyranoside (18)**: $BF_3{\cdot}OEt_2$ (1.20 mL, 9.5 mmol) was added to a solution of 2-acetoxy-D-glucal (**14**; 100.0 g, 303 mmol) and 4-methoxyphenol (40.0 g, 322 mmol) in benzene (1.20 L) under an atmosphere of nitrogen. After stirring at room temperature overnight, the reaction mixture was poured into saturated aqueous $NaHCO_3$. The separated organic layer was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resultant orange viscous oil (113.7 g) was recrystallized from methanol to afford **18** (56.9 g, 49%) as white crystals. M.p. 65°C; $[\alpha]_D^{21} = +150.8$ ($c = 1.02$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.03$ (s, 3H), 2.10 (s, 3H), 2.19 (s, 3H), 3.77 (s, 3H), 4.18–4.38 (m, 3H), 5.52 (ddd, $J = 10.0$, 2.0, 1.0 Hz, 1H), 5.56 (br s, 1H), 5.86 (d, $J = 2.0$ Hz, 1H), 6.83 (m, 2H), 7.03 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.5$, 20.76, 20.80, 55.5, 62.3, 65.1, 67.8, 93.7, 114.5, 116.0, 118.7, 145.7, 151.0, 155.5, 168.2, 170.1, 170.7 ppm; IR (KBr): $\tilde{\nu}_{max} = 2955$, 2839, 1748, 1508, 1373, 1214, 1034 cm^{-1}; elemental analysis calcd (%) for $C_{19}H_{22}O_9$: C 57.86, H 5.62; found: C 57.78, H 5.62.

***p*-Methoxyphenyl 3,4-dideoxy-α-D-*erythro*-hex-3-enopyranoside (21)**: A solution of lithium aluminum hydride (150 mg, 3.94 mmol) in THF (8.8 mL) was cooled to 0°C under an atmosphere of nitrogen. Glycoside **18** (500 mg, 1.32 mmol) in THF (1.0 mL) was then added dropwise to this solution over 1 h. After stirring at 0°C for 1 h, EtOAc (0.10 mL), water (0.15 mL), hexane (6.0 mL), 15% aqueous NaOH (0.15 mL), and water (0.45 mL) were sequentially added, and the reaction was stirred at room temperature overnight. The reaction mixture was then filtered though a pad of Super Cell, and the filter cake was washed with EtOAc and a 5:1 mixture of CH_2Cl_2 and methanol. Concentration of the filtrate under reduced pressure gave a crude solid (447 mg). This was purified by silica-gel chromatography (2:1 diethyl ether/hexane followed by diethyl ether as eluent) to afford **21** (277 mg, 84%) as white crystals. M.p. 140°C; $[\alpha]_D^{21} = +93.8$ ($c = 0.96$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.86$ (br s, 1H), 2.34 (d, $J = 12.0$ Hz, 1H), 3.58–3.78 (m, 2H), 3.80 (s, 3H), 4.31–4.45 (m, 2H), 5.61 (d, $J = 4.0$ Hz, 1H), 5.78 (dt, $J = 11.0$, 1.5 Hz,

1H), 5.92 (ddd, J=11.0, 4.0, 1.5 Hz, 1H), 6.85 (m, 2H), 7.06 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=55.6, 64.2, 64.7, 69.7, 96.9, 114.7, 118.1, 126.7, 128.7, 151.0, 155.4 ppm; IR (KBr): $\tilde{\nu}_{max}$=3336, 3228, 2950, 1509, 1457, 1228, 1038, 933, 828 cm^{-1}; elemental analysis calcd (%) for $C_{13}H_{16}O_5$: C 61.90, H 6.39; found: C 61.88, H 6.33.

***p*-Methoxyphenyl 6-*O*-(*tert*-butyldimethylsilyl)-3,4-dideoxy-α-D-*erythro*-hex-3-enopyranoside (22)**: *tert*-Butyldimethylsilyl chloride (10.0 g, 66.3 mmol) was added to a solution of diol **21** (25.0 g, 99 mmol), triethylamine (23.0 mL, 165 mmol), and 4-dimethylaminopyridine (0.80 g, 6.6 mmol) in CH_2Cl_2 (2.00 L). After stirring at room temperature overnight, additional triethylamine (8.0 mL, 57 mmol), 4-dimethylaminopyridine (0.25 g, 2.0 mmol), and *tert*-butyldimethylsilyl chloride (5.0 g, 33.3 mmol) were added, and stirring was continued overnight. The reaction mixture was concentrated to one-third of its volume and was then diluted with hexane. The resultant solution was washed with water, 1M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, brine, and dried (Na_2SO_4). Concentration under reduced pressure gave a viscous oil. This was dissolved in hexane, and upon cooling to −20°C, **22** (23.88 g) crystallized as a low-melting, white solid, which was filtered. The mother liquors were concentrated and then purified by recrystallization to provide additional product (3.18 g, total yield of 75%). M.p. 52°C; $[\alpha]_D^{24}$=+58.0 (c=1.03 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.05 (s, 6H), 0.88 (s, 9H), 2.36 (brd, J=12.0 Hz, 1H), 3.62 (dd, J=10.5, 6.0 Hz, 1H), 3.70 (dd, J=10.5, 5.5 Hz, 1H), 3.77 (s, 3H), 4.24 (m, 1H), 4.35 (m, 1H), 5.56 (d, J=4.5 Hz, 1H), 5.80–5.92 (m, 2H), 6.82 (m, 2H), 7.07 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=−5.50, −5.46, 18.1, 25.7, 55.6, 64.3, 65.2, 69.7, 96.9, 114.6, 118.0, 127.3, 127.9, 151.2, 155.2 ppm; IR (KBr): $\tilde{\nu}_{max}$=2929, 2858, 1509, 1473, 1219, 1096 cm^{-1}; elemental analysis calcd (%) for $C_{19}H_{30}O_5Si$: C 62.26, H 8.25; found: C 62.23, H 8.40.

***p*-Methoxyphenyl 6-*O*-(*tert*-butyldimethylsilyl)-2,3,4-trideoxy-4-(2,2,2-trichloroethoxycarbonylamino)-α-D-*erythro*-hex-2-enopyranoside (26a)**: Trichloroacetyl isocyanate (10.5 mL, 92.4 mmol) was added to a solution of **22** (28.2 g, 77.0 mmol) in CH_2Cl_2 (300 mL) at 0°C. After stirring at 0°C for 1 h, the reaction mixture was concentrated and the resultant residue was dissolved in methanol (100 mL). Water (100 mL) and potassium carbonate (16.1 g, 231 mmol) were added at 0°C, and the cooling bath was removed. After stirring at room temperature for 2.5 h, the reaction mixture was concentrated under reduced pressure to remove methanol. The resultant aqueous phase was extracted with CH_2Cl_2 and the combined organic layers were dried (Na_2SO_4) and concentrated to afford carbamate **23** (25.6 g), which was used for the next reaction without further purification.

Carbon tetrabromide (18.8 g, 56.6 mmol) in CH_2Cl_2 (30 mL) was added to a solution of carbamate **23** (8.27 g, 20.2 mol), triphenylphosphine (13.3 g, 50.5 mmol), and triethylamine (7.04 mL, 50.5 mmol) in CH_2Cl_2 (170 mL) at −40°C under an atmosphere of nitrogen. The reaction mixture was gradually warmed to 0°C over 30 min and was then stirred at 0°C for 1 h. 2,2,2-Trichloroethanol (11.6 mL, 0.12 mol) was introduced, and stirring was continued at 0°C for 2 h and then at room temperature overnight. The reaction mixture was washed with 1N HCl and aqueous saturated $NaHCO_3$, dried (Na_2SO_4), then concentrated under reduced pressure. The residue was purified by silica-gel chromatography (stepwise gradient of 15–30% diethyl ether/hexane as eluent) to furnish **26a** (10.1 g, 75% for the two steps). M.p. 77°C; $[\alpha]_D^{26}$=+108.5 (c=1.01 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.025 (s, 3H), 0.035 (s, 3H), 0.86 (s, 9H), 3.70–3.84 (m, 2H), 3.77 (s, 3H), 3.93 (m, 1H), 4.32 (t, J=9.0 Hz, 1H), 4.71 (d, J=12.0 Hz, 1H), 4.79 (d, J=12.0 Hz, 1H), 5.28 (brs, 1H), 5.52 (brs, 1H), 5.92–6.04 (m, 2H), 6.82 (m, 2H), 7.06 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=−5.64, −5.56, 18.2, 25.8, 46.6, 55.6, 63.5, 71.5, 74.5, 76.2, 94.0, 95.4, 114.5, 119.0, 126.6, 131.6, 151.3, 154.4, 155.2 ppm; IR (KBr): $\tilde{\nu}_{max}$=3804, 3746, 1718, 1735, 1719, 1541, 1508, 1216 cm^{-1}; elemental analysis calcd (%) for $C_{22}H_{32}Cl_3NO_6Si$: C 48.85, H 5.96, N 2.59; found: C 48.84, H 5.99, N 2.60.

***p*-Methoxyphenyl 2,3,4-trideoxy-4-(2,2,2-trichloroethoxycarbonylamino)-α-D-erythro-hex-2-enopyranoside (27)**: A solution of **26a** (318 mg, 0.59 mmol) and acetic acid (0.20 mL, 3.53 mmol) in THF (6.0 mL) cooled to 0°C was treated with tetrabutylammonium fluoride (1.0M solution in THF, 0.70 mL, 0.70 mmol). After stirring at 0°C for 1 h, the reaction mixture was poured into water and the aqueous layer was extracted with diethyl ether. The combined organic layers were washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica-gel chromatography (1:1 diethyl ether/hexane followed by diethyl ether as eluent) to furnish **27** (197 mg, 79%) as a low-melting solid. M.p. 38°C; $[\alpha]_D^{26}$=+71.9 (c=0.99 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.60 (t, J=7.0 Hz, 1H), 3.73–3.88 (m, 3H), 3.78 (s, 3H), 4.53 (brt, J=10.0 Hz, 1H), 4.69 (d, J=12.0 Hz, 1H), 4.84 (d, J=12.0 Hz, 1H), 5.08 (d, J=9.5 Hz, 1H), 5.63 (brs, 1H), 5.97–6.08 (m, 2H), 6.83 (m, 2H), 7.03 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=45.2, 55.5, 61.6, 71.5, 74.6, 93.8, 95.3, 114.6, 118.4, 127.1, 131.1, 151.0, 155.1, 155.2 ppm; IR (KBr): $\tilde{\nu}_{max}$=3738, 2944, 1735, 1560, 1508, 1216, 1102 cm^{-1}; elemental analyis calcd (%) for $C_{16}H_{18}Cl_3NO_6$: C 45.04, H 4.25, N 3.28; found: C 45.04, H 4.26, N 3.30.

Methyl 1-*O*-*p*-methoxyphenyl-2,3,4-trideoxy-4-(2,2,2-trichloroethoxycarbonylamino)-α-D-*erythro*-hex-2-enopyranosyluronate (29): DMSO (0.62 mL, 8.7 mmol) in CH_2Cl_2 (6.0 mL) was added to a solution of oxalyl chloride (0.56 mL, 6.42 mmol) in CH_2Cl_2 (18 mL) at −78°C under an atmosphere of nitrogen. After stirring at −78°C for 10 min, alcohol **27** (1.37 g, 3.21 mmol) in CH_2Cl_2 (6.0 mL) was added and stirring was continued for 1 h. Triethylamine (2.24 mL, 16.1 mmol) was introduced and the reaction mixture was stirred for 10 min at −78°C, and was then allowed to warm to 0°C. The resultant reaction mixture was poured into cold saturated aqueous NH_4Cl and the aqueous layer was extracted with diethyl ether. The combined organic layers were washed with brine, dried (Na_2SO_4), and then concentrated under reduced pressure to give the crude aldehyde **28** as a labile dark-brown liquid. This material was employed immediately without further purification in subsequent reactions.

Sodium chlorite (85%, 0.64 g, 7.1 mmol) was added portionwise to a solution of aldehyde **28**, 2-methyl-2-butene (2 drops), NaH_2PO_4 monobasic dihydrate (8.0 g) in a mixture of *tert*-butyl alcohol (32 mL) and water (8.0 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into saturated aqueous NH_4Cl and the aqueous layer was extracted with diethyl ether. The combined organic layers were dried (Na_2SO_4) and then concentrated under reduced pressure. Since the resultant crude carboxylic acid was not stable upon storage, the residue was immediately dissolved in methanol (32 mL) and was then treated at 0°C with a diethyl ether solution of diazomethane until the yellow color persisted. The excess diazomethane was quenched by dropwise addition of acetic acid until the color dissipated, and the resultant reaction mixture was concentrated under reduced pressure to give a yellow oil. Purification of the crude product by silica-gel chromatography (1:2 diethyl ether/hexane) provided **29** (1.02 g, 70% for three steps) as white crystals. M.p. 43°C; $[\alpha]_D^{26}$=+57.1 (c=1.43 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.76 (s, 3H), 3.77 (s, 3H), 4.49 (d, J=9.0 Hz, 1H), 4.62 (brt, J=9.0 Hz, 1H), 4.73 (brs, 2H), 5.25 (brd, J=9.0 Hz, 1H), 5.72 (brs, 1H), 6.02 (m, 2H), 6.83 (m, 2H), 7.03 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=47.1, 52.6, 55.4. 70.3, 74.5, 93.4, 95.2, 114.5, 118.3, 126.8, 130.4, 150.8, 154.2, 155.2, 169.3 ppm; IR (KBr): $\tilde{\nu}_{max}$=3343, 2955, 2837, 1745, 1508, 1440, 1216, 1036, 994, 828, 725 cm^{-1}; elemental analysis calcd (%) for $C_{17}H_{18}Cl_3NO_7$: C 44.91, H 3.99, N 3.08; found: C 45.08, H 4.07, N 3.14.

Methyl 1-*O*-acetyl-2,3,4-trideoxy-4-(2,2,2-trichloroethoxycarbonylamino)-D-*erythro*-hex-2-enopyranosyluronate (31): Silver(II) bis(hydrogen dipicolinate) (3.70 g, 8.00 mmol) was added to a solution of **29** (1.25 g, 2.77 mmol) in a mixture of acetonitrile (15.0 mL) and water (5.0 mL) at 0°C. The resultant suspension was vigorously stirred at 0°C for 15 min. The reaction mixture was diluted with CH_2Cl_2 (30 mL) to precipitate a white slurry, and the organic layer was decanted and then washed with water (40 mL). The aqueous layer was extracted with CH_2Cl_2 (about 40 mL) and the combined organic layers were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and filtered. Pyridine (4.0 mL), acetic anhydride (3.0 mL), and 4-dimethylaminopyridine (0.50 g, 4.10 mmol) were added to the filtrate. After standing at room temperature overnight, the resultant solution was concentrated under reduced pressure. The crude residue was purified by silica-gel chromatography (1:3 EtOAc/hexane) to afford acetyl glycoside **31** (760 mg, 71%) as an inseparable mixture of two anomers 3:1.

Fully protected cytosinine 32, its epimer 33, and the pyrrole by-product[31]: *N*,*O*-Bis(trimethylsilyl)acetamide (2.00 mL, 7.54 mmol) was slowly added to a slurry of N^4-*tert*-butylbenzoylcytosine (912 mg, 3.35 mmol) in THF (10.0 mL) under an atmosphere of nitrogen at room temperature. After stirring at room temperature for 30 min a homogeneous solution resulted. Trimethysilyl trifluoromethanesulfonate (TMSOTf, 0.14 mL,

0.78 mmol) was then added and stirring was continued for 30 min. The resultant solution was cooled to 0°C, and a solution of **31** (159 mg, 0.41 mmol) in THF (1.25 mL) was added. After stirring at 0°C for 2.0 h, the reaction mixture was diluted with CH_2Cl_2 and then poured into aqueous $NaHCO_3$. The resultant precipitate was filtered through Super Cell and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and concentrated. The crude residue (379 mg) was purified by silica-gel chromatography (1:7 and 1:1 of EtOAc/hexane followed by EtOAc) to afford a mixture of the two anomers, **32** and **33** (156 mg, 63%, **32**:**33** = 7:3, as determined by ^{1}H NMR analysis). The resultant mixture was roughly separated by silica-gel chromatography (100:1 CH_2Cl_2/MeOH), and **32** was further recrystallized from a mixture of EtOAc and hexane (three times) to afford pure **32** as pale-yellow crystals. Further purification of **33** was performed by preparative thin-layer chromatography (95:5 CH_2Cl_2/MeOH).

Compound **32**: m.p. 135°C; $[\alpha]_D^{27} = +102.6$ ($c = 0.28$ in $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 1.36$ (s, 9H), 3.72 (s, 3H), 4.43 (d, $J = 9.0$ Hz, 1H), 4.62 (dq, $J = 9.0$, 2.5 Hz, 1H), 4.79 (m, 2H), 5.95 (ddd, $J = 10.5$, 2.5, 2.0 Hz, 1H), 6.18 (dt, $J = 10.5$, 2.0 Hz, 1H), 6.66 (brs, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.63 (d, $J = 7.5$ Hz, 1H), 7.92 (d, $J = 8.5$ Hz, 1H), 8.23 ppm (d, $J = 7.5$ Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 31.5$, 36.0, 48.0, 53.3, 75.6, 77.4, 81.3, 97.1, 99.4, 126.9, 127.2, 129.3, 131.7, 134.5, 147.3, 156.5, 157.9, 158.3, 165.5, 170.4 ppm; IR (KBr): $\tilde{\nu}_{max} = 3421$, 2959, 1734, 1699, 1670, 1486, 1254 cm^{-1}; elemental analysis calcd (%) for $C_{25}H_{27}ClN_4O_7$: C 49.89, H 4.52, N 9.31; found: C 49.78, H 4.64, N 9.03.

Compound **33**: m.p. 137°C; $[\alpha]_D^{26} = -51.9$ ($c = 0.27$ in $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 1.35$ (s, 9H), 3.75 (s, 3H), 4.48 (m, 1H), 4.51 (d, $J = 5.5$ Hz, 1H), 4.80 (m, 2H), 6.02 (ddd, $J = 10.5$, 2.5, 2.0 Hz, 1H), 6.30 (ddd, $J = 10.0$, 3.5, 2.0 Hz, 1H), 6.77 (brs, 1H), 7.54–7.60 (m, 3H), 7.91 (m, 2H), 8.25 ppm (d, $J = 7.5$ Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): $\delta =$ 31.5, 36.0, 47.5, 53.2, 74.1, 75.6, 79.4, 97.1, 98.6, 126.9, 129.3, 131.7, 132.4, 148.1, 156.4, 158.2, 158.3, 165.5, 170.8 ppm; IR (KBr): $\tilde{\nu}_{max} = 3423$, 1741, 1654, 1256 cm^{-1}; elemental analysis calcd (%) for $C_{25}H_{27}ClN_4O_7$: C 49.89, H 4.52, N 9.31; found: C 49.88, H 4.70, N 9.10.

N,*O*-Bis(trimethylsilyl)acetamide (1.50 mL, 6.08 mmol) was slowly added to a slurry of N^4-*tert*-butylbenzoylcytosine (1.33 g, 4.88 mmol) in THF (12.0 mL) under an atmosphere of nitrogen at room temperature. After stirring at room temperature for 30 min a homogeneous solution resulted. Trimethysilyl trifluoromethanesulfonate (TMSOTf, 0.18 mL, 0.99 mmol) was then added and stirring was continued for a further 30 min. The resultant solution was cooled to 0°C and a solution of **31** (327 mg, 0.96 mmol) in THF (2.0 mL) was added. After stirring at 0°C for 3.5 h, the reaction mixture was diluted with CH_2Cl_2 and then poured into aqueous $NaHCO_3$. The resultant precipitate was filtered through Super Cell and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were then dried (Na_2SO_4) and concentrated. The crude residue (579 mg) was purified by silica-gel chromatography (1:7 and 1:1 of EtOAc/hexane followed by EtOAc) to afford the pyrrole by-product (60 mg, 20%),[31] as well as an anomeric mixture of **32** and **33** (319 mg, 55%) (α:β = 7:3, as determined by ^{1}H NMR analysis).

Improved synthesis for the preparation of dimethyl 3-benzyloxycarbonylaminoglutarate (34): A solution of dimethyl 3-oxoglutarate (20 mL, 136 mmol) and ammonium acetate (36.8 g, 476 mmol) in methanol (400 mL) was stirred over molecular sieves (3 Å pellets, 32 g) for 12 h. Sodium cyanoborohydride (10.8 g, 172 mmol) was added to the resultant reaction mixture and the solution was acidified to pH 3 by addition of methanolic HCl (prepared in another run by the addition of 60 mL of acetyl chloride to 800 mL of methanol at 0°C). The reaction mixture was then filtered, and the filtrate was concentrated under reduced pressure to afford the crude dimethyl 3-aminoglutarate hydrochloride which was dissolved in water (200 mL). The resultant aqueous solution was washed with diethyl ether and was then basified to pH 10 by the addition of solid sodium carbonate (31.8 g). To this aqueous solution was added diethyl ether (400 mL) and benzyl chloroformate (19.4 mL, 136 mmol). After vigorous stirring for 3 h at room temperature, *N*,*N*-dimethyl-1,3-propylamine (10.2 mL) was added to quench the excess benzyl chloroformate. The organic layer was separated and the aqueous layer was extracted with diethyl ether. The combined organic extracts were washed with 3N HCl, saturated aqueous $NaHCO_3$, brine, and dried (Na_2SO_4). Concentration under reduced pressure gave a residue (31.2 g) which was purified by silica-gel chromatography (1:3 EtOAc/hexane) to yield **34** as a colorless oil (30.29 g, 72%).

Benzyl-(3*S*)-3-benzyloxycarbonylamino-5-hydroxypentanamide (38): A diborane solution (1.08M solution in THF, 3.50 mL, 3.70 mmol) was added to a solution of carboxylic acid **35** (1.00 g, 3.70 mmol) in THF (30.0 mL) at −10°C under an atmosphere of nitrogen. After stirring at −10°C for 1 h the mixture was allowed to warm to room temperature, and stirring was continued at room temperature for 3 h. The resultant reaction mixture was quenched by the addition of MeOH, and was then concentrated under reduced pressure. The resultant residue was purified by silica-gel short column chromatography (eluted with EtOAc) to afford crude alcohol **36** (899 mg) as a colorless oil which was used in the next step without further purification. An analytically pure sample was prepared in another run and purified by chromatography to give **36** as a colorless oil. $[\alpha]_D^{27} = -34.0$ ($c = 0.75$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.64$ (m, 1H), 1.68 (brs, 1H), 1.80 (m, 1H), 2.56 (dd, $J = 16.0$, 5.0 Hz, 1H), 2.66 (dd, $J = 16.0$, 5.0 Hz, 1H), 2.90–3.00 (brs, 1H), 3.60–3.72 (brs, 1H), 3.68 (s, 3H), 4.22 (m, 1H), 5.10 (d, $J = 12.0$ Hz, 1H), 5.13 (d, $J = 12.0$ Hz, 1H), 5.62 (brd, $J = 9.0$ Hz, 1H), 7.30–7.39 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 37.0$, 38.6, 44.8, 51.7, 58.6, 66.9, 128.1, 128.2, 128.6, 136.3, 157.0, 172.2 ppm; IR (KBr): $\tilde{\nu}_{max} = 3355$, 1718, 1540, 1259, 1061 cm^{-1}; elemental analysis calcd (%) for $C_{14}H_{19}NO_5$: C 59.78, H 6.81, N 4.98; found: C 59.78, H 6.83, N 4.86.

Benzylamine (0.50 mL, 4.1 mmol) was slowly added to a solution of trimethylaluminum (1.0M solution in hexane, 1.70 mL, 1.70 mmol) in benzene (25.0 mL) at −10°C under an atmosphere of nitrogen. After being stirred at −10°C for 20 min, the mixture was allowed to warm to room temperature and was then stirred for a further 45 min. Methyl ester **36** (899 mg, crude) in benzene (1.0 mL) was then added to this solution at 0°C. After being stirred at 0°C for 20 min, the reaction mixture was allowed to warm to room temperature and was stirred for a further 2 h. The reaction was then quenched by the addition of MeOH. The resultant mixture was filtered through a pad of Super Cell and the filtrate was concentrated under reduced pressure to give a residue. This was purified by silica-gel column chromatography (eluted with 95:5 CH_2Cl_2/MeOH) to afford benzylamide **38** (1.10 g, 83% for two steps) as pale-yellow crystals. M.p. 127°C); $[\alpha]_D^{24} = -16.4$ ($c = 1.02$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 1.73$ (m, 2H), 2.45 (d, $J = 7.0$ Hz, 2H), 3.59 (d, $J = 6.0$ Hz, 1H), 3.61 (d, $J = 6.0$ Hz, 1H), 4.12 (brqn, $J = 7.0$ Hz, 1H), 4.31 (d, $J = 15.0$ Hz, 1H), 4.35 (d, $J = 15.0$ Hz, 1H), 5.05 (s, 2H), 7.18–7.35 ppm (m, 10H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 38.5$, 42.5, 44.1, 47.6, 59.7, 67.4, 128.3, 128.7, 128.9, 129.1, 129.55, 129.62, 138.4, 139.9, 158.4, 173.3 ppm; IR (KBr): $\tilde{\nu}_{max} = 3309$, 1691, 1645, 1542, 1272 cm^{-1}; elemental analysis calcd (%) for $C_{20}H_{24}N_2O_4$: C 67.40, H 6.79, N 7.86; found: C 67.40, H 6.68, N 7.97.

Benzyl-(3*S*)-3-benzyloxycarbonylamino-5-(*N*-methyl-2-nitrobenzensulfonylamino)pentanamide (39): Diethyl azodicarboxylate (0.40 mL, 2.55 mmol) was added to a solution of **38** (324 mg, 0.91 mmol), *N*-methyl-2-nitorobenzenesulfonamide (275 mg, 1.27 mmol), and tri-*n*-butylphosphine (0.64 mL, 2.55 mmol) in THF (15 mL) at 0°C. The reaction mixture was warmed to room temperature and was then stirred for 2 h. Additional tri-*n*-butylphosphine (0.64 mL, 2.55 mmol) and diethylazodicarboxylate (0.40 mL, 2.55 mmol) were introduced at room temperature. The resultant solution was stirred for 20 min and was then concentrated under vacuum. The residue was purified by silica-gel column chromatography (eluted with 1:1 EtOAc/hexane followed by 3:1 EtOAc/hexane) to afford **39** (421 mg, 83%) as a pale-yellow solid. M.p. 73°C; $[\alpha]_D^{22} = -13.6$ ($c = 1.01$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.70$ (brs, 1H), 1.82 (m, 1H), 2.01 (m, 1H), 2.55 (m, 2H), 2.88 (s, 3H), 3.16–3.38 (m, 2H), 3.97 (m, 1H), 4.40 (m, 2H), 5.07 (s, 2H), 6.01 (d, $J = 7.0$ Hz, 1H), 6.22 (brs, 1H), 7.24–7.38 (m, 10H), 7.58–7.74 (m, 3H), 7.90 ppm (dd, $J = 7.0$, 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 31.7$, 34.5, 39.2, 43.4, 46.5, 47.1, 66.5, 124.1, 127.5, 127.8, 127.9, 128.1, 128.5, 128.7, 130.7, 131.7 (2C), 133.7, 136.6, 138.1, 148.3, 156.1, 170.7 ppm; IR (KBr): $\tilde{\nu}_{max} = 3309$, 1700, 1652, 1542, 1373, 1350, 1244, 1165 cm^{-1}; elemental analysis calcd (%) for $C_{27}H_{26}N_4O_7S$: C 58.47, H 5.45, N 10.10; found: C 58.47, H 5.38, N 10.11.

Benzyl-(3*S*)-3-benzyloxycarbonylamino-5-(*N*-methyl-bis-*tert*-butoxycarbonylguanidyl)pentanamide (41): Thiophenol (93 μL, 0.91 mmol) was added to a solution of **39** (421 mg, 0.76 mmol) and cesium carbonate (750 mg, 2.30 mmol) in acetonitrile (13 mL). The heterogeneous reaction mixture was then vigorously stirred at room temperature for 2 h. Addi-

tional thiophenol (39 μL, 0.38 mmol) was introduced, and after stirring for a further 2 h, a further portion of thiophenol (39 μL, 0.38 mmol) was added and stirring was continued until TLC analysis showed the absence of starting material. The resultant reaction mixture was diluted with CH_2Cl_2 and then filtered through a pad of Super Cell, and the filter cake was washed with EtOAc. The filtrate was concentrated to give a residue (502 mg) which was subsequently dissolved in DMF (15 mL). *N,N*-Di-(*tert*-butoxycarbonyl)-*S*-methylisothiourea (232 mg, 0.80 mmol) and triethylamine (0.24 mL, 1.70 mmol) were then added to this solution. The reaction mixture was cooled to 0 °C and then treated with mercuric chloride (228 mg, 0.84 mmol). After stirring at 0 °C for 2 h, the mixture was diluted with EtOAc and then filtered though a pad of Super Cell. The filtrate was washed with water and the aqueous layer was extracted with EtOAc. The combined extracts were washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica-gel column chromatography (1:1 followed by 2:1 EtOAc/hexane) to furnish **41** (412 mg, 89 %) as a colorless gum. $[\alpha]_D^{23} = +14.6$ ($c = 0.59$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 1.43 (s, 18H), 1.72 (brs, 1H), 1.79 (m, 1H), 2.01 (m, 1H), 2.49 (dd, $J =$ 14.0, 6.0 Hz, 1H), 2.69 (br dd, $J = 14.0$, 5.0 Hz, 1H), 2.94 (s, 3H), 3.56 (brs, 2H), 3.92 (m, 1H), 4.37 (dd, $J = 14.0$, 6.0 Hz, 1H), 4.45 (dd, $J = 14.0$, 6.0 Hz, 1H), 5.05 (s, 2H), 6.50 (brs, 1H), 6.85 (brs, 1H), 7.20–7.36 (m, 10H), 9.88 ppm (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 28.0$, 30.7, 36.8, 39.8, 43.3, 47.0, 47.2, 66.3, 79.6, 81.9, 127.2, 127.6, 127.8, 128.2, 128.3, 128.5, 136.7, 138.3, 150.6, 155.8, 156.1, 161.8, 170.8 ppm; IR (KBr): $\tilde{\nu}_{max} =$ 3332, 2928, 1700, 1647, 1610, 1541, 1508, 1298 cm^{-1}; elemental analysis calcd (%) for $C_{47}H_{69}N_5O_{11}$: C 62.83, H 7.41, N 11.45; found: C 62.78, H 7.42, N 11.47.

Benzyl-(3*S*)-3-benzyloxycarbonyl-*tert*-butoxycarbonylamino-5-(*N*-methyl-tris-*tert*-butoxycarbonylguanidyl)pentanamide (44): Di-*tert*-butyldicarbonate (10.90 g, 50 mmol) was added in one portion to a solution of **41** (5.10 g, 8.34 mmol) and 4-dimethylaminopyridine (1.53 g, 12.5 mmol) in THF (50 mL). After stirring at room temperature overnight, the reaction mixture was diluted with diethyl ether and then washed with saturated aqueous $NaHCO_3$ and brine, and dried (Na_2SO_4). Concentration under reduced pressure gave a residue which was purified by silica-gel column chromatography (1:2 EtOAc/hexane) to provide **44** (5.08 g, 67 %) as a viscous syrup. $[\alpha]_D^{27} = +3.44$ ($c = 0.39$ in $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 27.81, 27.83, 27.86, 27.9, 28.1, 29.7, 30.8, 35.4, (36.5), (42.2), 42.3, 47.2, (47.8), 48.1, (52.7), 52.8, 68.4, (68.6), 79.2, (79.3), 83.0, (83.1), 83.3, (83.4), 83.5, 147.8, (148.0), 149.3, (150.8), (152.6), 152.8, (154.1), 154.4, (158.9), 159.1, (173.1), 173.3 ppm; IR (KBr): $\tilde{\nu}_{max} =$ 2980, 1803, 1735, 1706, 1609, 1456 cm^{-1}; elemental analysis calcd (%) for $C_{47}H_{69}N_5O_{11}$: C 61.89, H 7.63, N 7.68; found: C 61.95, H 7.69, N 7.65.

Benzyl-(3*S*)-3-benzyloxycarbonyl-5-(*N*-methyl-tris-*tert*-butoxycarbonyl-guanidyl)pentanamide (45): A solution of **44** (5.10 g, 5.59 mmol) and palladium hydroxide on carbon (Pearlman's catalyst, 0.50 g) in ethanol (150 mL) was stirred vigorously for 5 h. The reaction mixture was filtered though a pad of Super Cell, the filter cake was washed with diethyl ether, and the filtrate was washed with saturated aqueous $NaHCO_3$ and brine, and then dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica-gel column chromatography (1:3 and 1:2 EtOAc/hexane) to afford **45** (3.37 g, 77 %) as a white amorphous solid. M.p. 52 °C; $[\alpha]_D^{24} = -4.30$ ($c = 1.09$ in $CHCl_3$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 27.6, 27.7, 27.9, 28.1, 28.2, 30.3, (33.4), 35.6, (36.5), 42.8, (46.2), 46.4, 47.0, (47.6), 48.0, 77.2, 78.8, 79.1, (79.2), 83.4, (83.6), (127.0), (127.1), 127.2, 128.2, (137.8), 138.0, 147.76, 147.80, (148.0), (148.1), 149.6, (150.9), 152.9, (155.4), 155.5, 158.9, (173.6), 173.9 ppm; IR (KBr): $\tilde{\nu}_{max} =$ 3395, 2980, 1735, 1713, 1609, 1285 cm^{-1}; elemental analysis calcd (%) for $C_{39}H_{63}N_5O_{11}$: C 60.21, H 8.16, N 9.00; found: C 60.22, H 8.21, N 9.01.

Methyl (3*S*)-3-benzyloxycarbonyl-5-(*N*-methyl-tris-*tert*-butoxycarbonyl-guanidyl)pentanoate (46): 1,1,3,3-Tetramethylguanidine (0.15 mL, 1.20 mmol) was added to a solution of **45** (793 mg, 1.02 mmol) in methanol (10 mL). After being stirred at room temperature for 3 h, the reaction mixture was diluted with diethyl ether and washed successively with water, 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure, and the resultant residue was purified by silica-gel chromatography (1:1 EtOAc/hexane) to afford **46** (533 mg, 87 %) as a colorless oil. $[\alpha]_D^{28} = -3.08$ ($c = 1.37$ in $CHCl_3$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 27.8, 28.0, 28.3, 30.0, 35.7, (36.8), 38.9, 45.8, (47.6), 47.9, 51.5, 79.4, 83.7, 147.8, (147.9), 149.9, 155.6, 158.9, 172.1 ppm; IR (KBr): $\tilde{\nu}_{max} =$ 2980, 1802, 1718, 1609, 1286, 1159, 1103 cm^{-1}; elemental analysis calcd (%) for $C_{22}H_{23}NO_3$: C 55.80, H 8.36, N 9.30; found: C 55.72, H 8.29, N 9.26.

Tetra-Boc-blastidic acid (47): Lithium hydroxide monohydrate (153 mg, 1.22 mmol) was added to a solution of **46** (205 mg, 0.34 mmol) in a mixture of THF (2.6 mL) and water (0.9 mL). After stirring for 3 h at room temperature, the reaction mixture was poured into cold saturated aqueous 1 M $KHSO_4$ and extracted with EtOAc. The resultant organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give carboxylic acid **47** (198 mg, 97 %) as a fine white powder. M.p. 169 °C; $[\alpha]_D^{25} = -4.52$ ($c = 1.17$ in $CHCl_3$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 27.8, 27.9, 28.0, 28.2, 30.0, 35.7, (36.8), (37.0), 39.0, 45.7, 47.9, 79.4, 79.9, 83.8, 83.9, 147.77, 147.84, 150.0, 155.8, 158.9, 175.3 ppm; IR (KBr): $\tilde{\nu}_{max} =$ 3448, 1718, 1637, 1283, 1158, 1119 cm^{-1}; elemental analysis calcd (%) for $C_{27}H_{48}N_4O_{10}$: C 55.09, H 8.22, N 9.52; found: C 55.11, H 8.08, N 9.50.

Blastidic acid dihydrochloride (51): Trifluoroacetic acid (0.10 mL) was added to a solution of carboxylic acid **47** (62 mg, 0.11 mmol) in CH_2Cl_2 (0.5 mL) at room temperature. After stirring at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was quickly dissolved in a few drops of 3 N aqueous HCl and then evaporated under vacuum. This was repeated two more times, then the resultant residue was dissolved in hot ethanol and filtered through a pad of Super Cell. A few drops of acetone were added to the filtrate and the resultant solution was allowed to stand at room temperature to afford crude **51** as a white solid (19 mg, 69 %). The crude solid was dissolved in a few drops of water, loaded onto a short column of IRA-410 (5 mL, wet volume), and then eluted with water. The eluent was immediately passed through a short column of IRC-50 (6 mL, wet volume) and the column was washed with water (15 mL). Elution with 0.5 N HCl afforded blalstidic acid dihydrochloride **51** (14.8 mg, 54 %), which was further recrystallized from ethanol to furnish a white crystalline solid (10.3 mg, 37 %) [m.p. 190–195 °C (decomp)]. $[\alpha]_D^{20} = +13.3$ ($c = 0.52$ in H_2O); ^{1}H NMR (300 MHz, D_2O): $\delta = 2.05$ (m, 2H), 2.69 (dd, $J =$ 17.5, 7.5 Hz, 1H), 2.82 (dd, $J =$ 17.5, 4.5 Hz, 1H), 3.04 (s, 3H), 3.49 (m, 2H), 3.65 ppm (qd, $J =$ 7.0, 4.5 Hz, 1H); ^{13}C NMR (75 MHz, D_2O): $\delta = 29.9$, 36.4, 36.6, 46.7, 47.4, 157.5, 174.6 ppm; IR (KBr): $\tilde{\nu}_{max} =$ 3147, 2827, 1717, 1648, 1628, 1488, 1414, 1191 cm^{-1}; HRMS (FAB): m/z: calcd for $C_7H_{17}N_4O_2$ $[M+H]^+$: 189.1352; found: 189.1337; elemental analysis calcd (%) for $C_7H_{18}Cl_2N_4O_2$: C 32.19, H 6.95, N 21.45; found: C 32.18, H 7.09, N 21.42.

Deprotection of the Troc group in 32: Freshly prepared Cd/Pb (10 %, 1.50 g) was added in one portion to a solution of **32** (189 mg, 0.31 mmol) in a mixture of THF (8.0 mL) and 1 M ammonium acetate buffer (2.0 mL, pH 7). After vigorous stirring at room temperature for 30 min, the reaction mixture was diluted with CH_2Cl_2 and then filtered through a pad of Super Cell. The filtrate was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated under reduced pressure to give a residue which was purified by silica-gel chromatography (100:1 CH_2Cl_2/MeOH) to afford **53** (109 mg, 93 %, calculated based on consumed **32**) and recovered **32** (26 mg, 13 %). $[\alpha]_D^{28} = +87.6$ ($c = 1.15$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.35$ (s, 9H), 3.80 (dq, $J = 9.0$, 2.5 Hz, 1H), 3.82 (s, 3H), 4.12 (d, $J = 9.0$ Hz, 1H), 5.79 (ddd, $J = 10.0$, 2.5, 2.0 Hz, 1H), 6.16 (dt, $J = 10.0$, 2.0 Hz, 1H), 6.72 (brs, 1H), 7.53 (d, $J = 8.5$ Hz, 2H), 7.50–7.58 (m, 1H), 7.75 (d, $J = 7.5$ Hz, 1H), 7.84 ppm (d, $J = 8.5$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 30.9$, 35.0, 47.1, 52.5, 80.0, 80.1, 97.6, 125.4, 126.0, 127.6, 130.0, 136.1, 145.0, 154.9, 157.2, 162.7, 169.3 ppm; IR (KBr): $\tilde{\nu}_{max} =$ 3421, 2963, 1670, 1488, 1257 cm^{-1}; HRMS (FAB): m/z: calcd for $C_{22}H_{27}N_4O_5$ $[M+H]^+$: 427.1981; found: 427.1974.

Coupling of 53 and 47 using the BOP method in the presence of HOBt: BOP (132 mg, 0.30 mmol) and HOBt (40 mg, 0.30 mmol) were added to a solution of **47** (113 mg, 0.19 mmol), **53** (41 mg, 0.096 mmol), and diisopropylethylamine (0.15 mL, 0.86 mmol) in CH_2Cl_2 (5.0 mL). After being stirred at room temperature for 1.5 h, additional BOP (23 mg, 0.052 mmol), HOBt (8.0 mg, 0.059 mmol), and **47** (23 mg, 0.039 mmol) were introduced. After a further 5 h, the solution was diluted with saturated aqueous NaCl and then extracted with EtOAc. The combined organic layers were washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), and then concentrated under re-

duced pressure to give a residue which was purified by silica-gel chromatography (100:1 CH_2Cl_2/MeOH) to afford **54** (70 mg, 73%) as a pale-yellow powder. M.p. 146°C; $[\alpha]_D^{30} = +52.3$ ($c = 0.39$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.35$ (s, 9H), 1.42 (s, 9H), 1.46 (s, 9H), 1.49 (s, 9H), 1.51 (s, 9H), 1.71 (brs, 1H), 2.23 (brs, 1H), 2.46 (brs, 1H), 2.81 (brd, 1H), 2.87 (d, 3H), 3.27 (brs, 1H), 3.74 (s, 3H), 3.76–3.93 (m, 2H), 4.43 (d, $J = 9.0$ Hz, 1H), 4.96 (tq, $J = 9.0$, 2.5 Hz, 1H), 5.81 (dt, $J = 10.0$, 2.0 Hz, 1H), 6.11 (brd, $J = 10.0$ Hz, 1H), 6.71 (brs, 1H), 7.52 (d, $J = 8.5$ Hz, 2H), 7.57 (brs, 1H), 7.71(d, $J = 8.5$ Hz, 1H), 7.80 (d, $J = 7.5$ Hz, 1H), 7.85 ppm (d, $J = 8.5$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 27.8$, 27.9, 28.0, 28.3, 30.9, 34.8, 35.0, 39.6, 44.7, 46.4, 47.1, 52.6, 76.1, 77.2, 79.0, 79.6, 80.7, 84.0, (84.2), 97.7, 125.3, 125.8, 126.0, 127.7, 129.4, 130.0, 133.4, 145.2, 148.0, 150.0, 155.6, 157.2, 158.8, 162.9, 168.4, 171.6 ppm; IR (KBr): $\tilde{\nu}_{max} = 3569$, 2971, 1698, 1654, 1256, 1116 cm^{-1}; elemental analysis calcd (%) for $C_{49}H_{72}N_8O_{14}$: C 59.02, H 7.28, N 11.24; found: C 58.92, H 7.24, N 11.17.

Total synthesis of blasticidin S (1): Triethylamine (0.05 mL, 0.36 mmol) was added to a solution of **54** (23 mg, 0.023 mmol) in MeOH (1.0 mL) at room temperature, and stirring was continued overnight. Water (0.40 mL, 0.02 mol) was added and the solution was left at room temperature overnight. The resultant reaction mixture was concentrated under reduced pressure to afforded the crude carboxylate (13 mg), which was dissolved in CH_2Cl_2 (1.0 mL) and then treated with TFA (0.10 mL, 1.3 mmol) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in 3N aqueous HCl (0.2 mL, 0.2 mmol) and then concentrated under vacuum. This was repeated three times. The resultant hydrochloride was dissolved in distilled water and then passed through a column of IRA-410. The effluent that showed UV absorption was collected and concentrated. The resultant residue was dissolved in ethanol and then precipitated by addition of diethyl ether to afford white crystals of blasticidin S (**1**) (8 mg, 85%) [m.p. 238–239°C (dec.)]. $[\alpha]_D^{27} = +70.5$ ($c = 0.33$ in H_2O); ^{1}H NMR (300 MHz, 1N DCl): $\delta = 2.08$ (m, 2H), 2.76 (dd, $J = 16.0$, 7.0 Hz, 1H), 2.82 (dd, $J = 16.0$, 5.0 Hz, 1H), 3.06 (s, 3H), 3.51 (m, 2H), 3.71 (qn, $J = 6.0$ Hz, 1H), 4.54 (d, $J = 8.0$ Hz, 1H), 4.87 (dq, $J = 8.0$, 2.5 Hz, 1H), 5.98 (ddd, $J = 10.0$, 2.5, 2.0 Hz, 1H), 6.27 (d, $J = 8.0$ Hz, 1H), 6.28 (dt, $J = 10.0$, 2.0 Hz, 1H), 6.60 (q, $J = 2.0$ Hz, 1H), 7.85 ppm (d, $J = 8.0$ Hz, 1H); ^{13}C NMR (100 MHz, 1N DCl): $\delta = 29.9$, 36.8, 37.2, 45.2, 47.3, 47.5, 75.5, 80.2, 96.4. 125.3, 134.1, 146.4, 149.1, 157.3, 160.1, 172.0, 172.3 ppm; IR (KBr): $\tilde{\nu}_{max} = 3221$, 1729, 1679, 1655, 1431, 1197 cm^{-1}; HRMS (FAB): m/z: calcd for $C_{17}H_{27}N_8O_5$ $[M+H]^+$: 423.2104; found: 423.2119.

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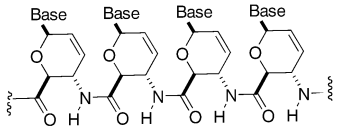
[11] We recognized structural similarities between cytosinine **3** and peptide nucleic acid (PNA); this led us to propose a new type of PNA as shown below. Our research efforts into the synthesis and characterization of this new PNA is now in progress. For PNA, see the following reference: P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624–630.



PNA (i)

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[17] The acyl group on the cytosine N^4-nitrogen (PG^2 in **8**) appeared to be very fragile. For example, gradual cleavage of the *tert*-butylbenzoyl group in **32** occurred even in neutral methanol. For other examples, see: Y. Isido, N. Nakazaki, N. Sakairi, *J. Chem. Soc. Perkin Trans. 1* **1977**, 657–660.

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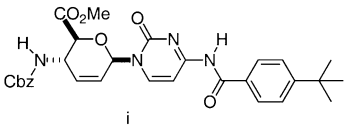
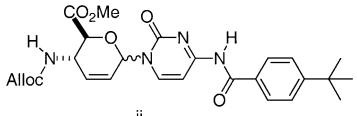
[23] Although we initially employed **26b** as an intermediate, this route was finally abandoned due to the difficulties associated with deprotection of the Cbz group in **i**. In the case of **26c**, separation of the anomeric mixture, as well as deprotection of **ii** proved to be seriously difficult.

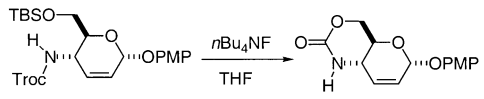


i



ii

[24] A. B. Smith, G. R. Ott, *J. Am. Chem. Soc.* **1996**, *118*, 13095–13096. Attempted removal of the TBS group in **26a** in the absence of acetic acid led to the formation of cyclic carbamate **i** as the major product.



26a i

[25] Oxidative removal of the *p*-methoxyphenyl group in **29** with ceric ammonium nitrate (CAN) gave the desired product in poor yields. The silver(II) reagent which was finally used successfully can also be used to deprotect PMB ethers as well as PMB amides. Further details will be reported in due course.

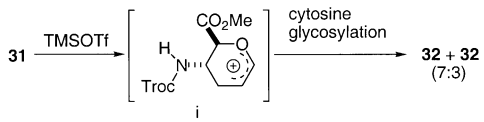
[26] Since we observed gradual decomposition of **31** during purification on silica-gel chromatography, we did not attempt to separate the α- and β-isomers. Moreover, this material also slowly decomposed when stored in a refrigerator at −20 °C.

[27] D. Will, G. Breipohl, D. Langner, J. Knolle, E. Uhlmann, *Tetrahedron* **1995**, *51*, 12069–12082.

[28] Upon addition of Lewis acids, such as $SnCl_4$ or $BF_3{\cdot}OEt_2$, into the reaction mixture that contained the silylated cytosine, we frequently observed the formation of a white precipitate. This appeared to be a σ-complex of the silylated base and Lewis acid. For such σ-complexes see: a) H. Vorbrüggen, G. Höfle, *Chem. Ber.* **1981**, *114*, 1256–1268; b) H. Vorbrüggen, C. Ruh-Pohlenz, *Org. React.* **2000**, *55*, 1.

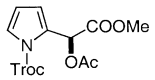
[29] a) G. E. Hilbert, T. B. Johnson, *J. Am. Chem. Soc.* **1930**, *52*, 2001–2007; b) H. Vorbrüggen, K. Krolikiewicz, B. Bennua, *Chem. Ber.* ***1*981**, *114*, 1234–1255; c) T. Ueda, Watanabe, S.-I. *Chem. Pharm. Bull.* **1985**, *33*, 3689–3695.

[30] Cytosine N-glycosylation in the current case might involve a delocalized cation such as **i**, which is also assumed to be an intermediate in the Ferrier reaction. In the Ferrier glycosylation using alcohols and carbon nucleophiles, formation of α-glycosides is usually preferential. Whilst the *α*- and *β*-selectivity of O- and C-Ferrier type glycosylations seems to be governed by a transition state that is stabilized by anomeric effects, we feel that the transition state for N-glycosylation in this case is not so strongly stabilized by anomeric effects. Therefore, the moderate selectivity observed in this reaction (7:3) might be the result of steric effects. In particular, the bulky silylated cytosine derivative could approach the probable intermediate **i** from the relatively less hindered β-face. We discuss the selectivity of the Ferrier N-glycosylation based on kinetic control. However, it should be noted that one of the referees cleverly pointed out an alternative explanation based on thermodynamics in which an *exo*-anomeric effect is operative in the preferred formation of the equatorial N-glycoside.



i

[31] α-Substituted pyrrole **i** was isolated as the major by-product. An authentic sample was also prepared by hydrolysis of **29** using 3 N HCl and subsequent acetylation (Ac_2O, pyridine). The mechanism to form **i** is unclear.



i

[32] Diester **34** was prepared using a modified procedure reported by Crossley, see Experimental Section and reference: M. J. Crossley, M. L. Fisher, J. J. Potter, P. W. Kuchel, M. J. York, *J. Chem. Soc. Perkin Trans. 1* **1990**, 2363–2369.

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[35] a) A. Basha, M. Lipton, S. M. Weinreb, *Tetrahedron Lett.* **1977**, *18*, 4171–4174; b) M. Lipton, A. Basha, S. M. Weinreb, *Org. Synth. Coll.* **1988**, *6*, 492–495.

[36] T. Fukuyama, C-K. Jow, M. Cheung, *Tetrahedron Lett.* **1995**, *36*, 6373–6374.

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[38] D. L. Flynm, R. E. Zelle, D. A. Grieco, *J. Org. Chem.* **1983**, *48*, 2424–2426.

[39] L. Grehn, K. Gunnarsson, U. Ragnarsson, *Acta Chem. Scand.* **1987**, *B41*, 18–23. Although one-step hydrolysis of **45** using Evans' protocol (H_2O_2, LiOH)[34] provided carboxylic acid **47**, the two-step procedure which involved methanosysis followed by saponification gave higher and more reliable yields.

[40] An authentic sample of pseudoblastidone (**52**) was prepared by treatment of blastidic acid dihydrochloride with base (0.5 N NaOH, pH 10.0). See ref. [8d].

[41] We would like to thank Prof. T. Kondo, Nagoya University, for his generous gift of blastidic acid dihydrochloride and cytosinine dihydrochloride. These were prepared by hydrolysis of natural **1**.

[42] L. F. Fieser, M. Fieser, *Reagents for Organic Synthesis, Vol. 1*, Wiley, **1967**, p. 1276.

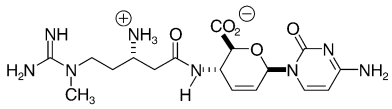
[43] a) P. Knochel, M. C. P. Yeh, S. C. Berk, J. Talbert, *J. Org. Chem.* **1988**, *53*, 2390–2392; b) K. Takai, T. Kakiuchi, K. Utimoto, *J. Org. Chem.* **1994**, *59*, 2671–2673.

[44] Q. Dong, E. Anderson, M. A. Ciufolini, *Tetrahedron Lett.* **1995**, *36*, 5681–5682. The use of freshly prepared Cd/Pb turned out to be essential. Moreover, it should be noted that the yields were time-dependent. Deprotection over an extended period of time led to side reactions to afford highly polar products which were quite difficult to isolate. Most probably, the by-products may arise from hydrolysis of the *tert*-butylbenzoyl group.

[45] B. Castro, J.-R. Dormoy, G. Evin, C. Selve, *Tetrahedron Lett.* **1975**, *16*, 1219–1222.

[46] a) W. König, R. Geiger, *Chem. Ber.* **1970**, *103*, 788–798; b) W. König, R. Geiger, *Chem. Ber.* **1970**, *103*, 2024–2033.

[47] Blasticidin S purified by this method is considered to be a zwitterion as depicted in **i**.



i

[48] Since the salt-free blasticidin S gradually decomposes to give cytomycin **55**, NMR spectra of our synthetic sample were immediately measured in 1 N DCl after purification, since this provides stable blasticidin S hydrochloride.

[49] The overall yield, including the cytosine N-glycosylation step (**31**→**32**), is calculated to be 44% (63% multiplied by 0.7).

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