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A SYNTHESIS OF NIKKOMYCIN Z: IMPROVED SYNTHESIS AND PROTECTION OF THE PYRIDYL γ-HYDROXY-α-AMINOBUTANOIC ACID COMPONENT‡

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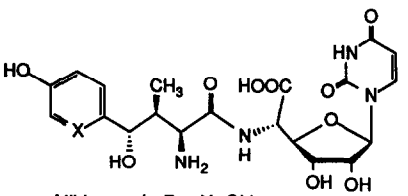
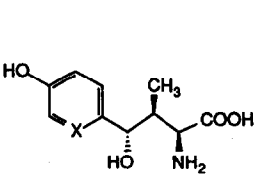
Abstract: The first synthesis of nikkomycin Z is described. An improvement of the isoxazoline-based methodology[6,12] and an effective method for protection of **1b** were devised. The application of oxalyditriazole **(23)** for peptide coupling proved most effective in completing the synthesis. A new and efficient synthesis of the prerequisite pyridyl *E*-olefin **(5)** is also described.

The nikkomycin family of antifungal antibiotics inhibit fungal cell wall chitin biosynthesis.[1] In view of increased demands for treating opportunistic fungal infections, chitin synthetase inhibition appears to be an attractive approach for the design of safer antifungal agents. Nikkomycins X and Z have shown efficacy in a disseminated candidiasis model in mice[2] as well as systemic *Coccidioides immitis* and *Blastomyces dermatitidis* infections.[3] These are the first examples of a chitin synthetase inhibitor showing activity in systemic animal infections.

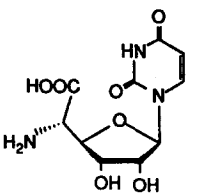
A number of nikkomycins have been characterized,[4] and some approaches to the synthesis of the two principal aromatic butanoic acid components **1a**[5,6] and **1b**[7] have been reported earlier.[6,8-10] Barrett[11] recently described a highly stereoselective synthesis of the nikkomycin B aryl butanoic acid component **1a**, a homotyrosine analog. In accord with our own observations, it was notable that chemistry useful in the phenyl series (cf. nikkomycin B) generally failed[11] when applied to analogous pyridyl series (cf. nikkomycin X and Z) compounds. This prompted us to develop a practical synthesis and protection of selected stereoisomers of the pyridyl aminobutanoic acid component **1b** as well as its coupling to uracil polyoxin C (**2**, UPoC), culminating in the synthesis of nikkomycin Z which we now describe.



Nikkomycin B_Z: X=CH
Nikkomycin Z : X=N



1a : X = CH
1b : X = N



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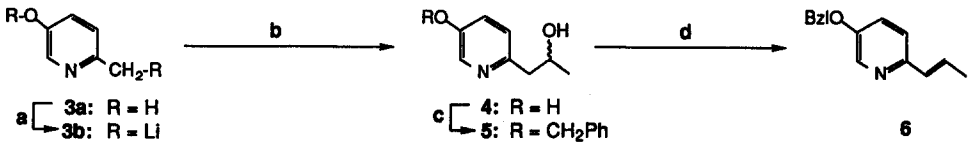
When we initially required all eight possible isomers of **1b**, the nitrile oxide cycloaddition/isoxazoline reduction methodology of König[12] seemed most suitable. Our subsequent focus on 3*R**,4*R** isomers led us to concentrate on the *trans*-olefin **6** (or analog) from which they evolved. Since the reported[10] 7-step synthesis of the acetate corresponding to **6** (17% yield) proved impractical, a more efficient synthesis was imperative. Thus, the deep red dianion **3b**[13] reacted with acetaldehyde to give **4**.[14] Selective benzylation[15] of **4** followed by dehydration gave **6** in overall 55-60% yields in 3 steps (Scheme I).

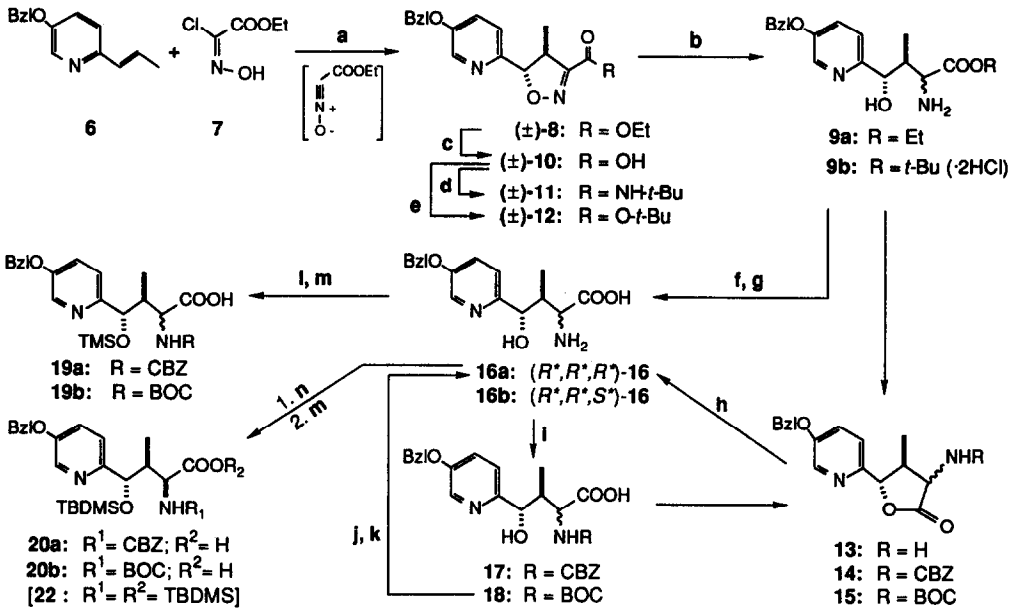
SCHEME 1



Reagents . **a:** *sec*-BuLi (2 eq.), THF, -20°C. **b:** excess CH_3CHO, -20 → 20°C; H_3O^+ (85%). **c:** NaH, BzlBr, DMF (98%). **d:** KOH (2 eq.), hydroquinone, DMSO,160°C,0.5hr (70%).

With sufficient **6** in hand we were unable to reproduce the nitrile oxide cycloaddition to obtain **8** even in the reported moderate yields.[6,8,10] It appeared that the relatively low nucleophilicity of **6** (cf. phenyl analogs) could be overcome by high dilution of the nitrile oxide, avoiding the undesired self condensation of the latter. This was best achieved by very slow infusion of aqueous Na_2CO_3 into an ethereal solution of **7** and the olefin **6**. In this manner isoxazoline (*R*,R**)-**8**[16] could be obtained consistently in over 70% yields (Scheme 2).

SCHEME 2



Reagents. **a:** 0.05M 5/Et_2O, xs 7, slow addition of aq. Na_2CO_3 (70%). **b:** 8 or 12, Zn-Cu, HOAc, 22-27°C, 6 hr./50 mmol; HCl (79%). **c:** KOH, THF-H_2O (95%). **d:** Im_2CO, DMF, r.t.; *t*-$BuNH_2$ (70%). **e:** Im_2CO, DMF, r.t.; *t*-BuOK, *t*-BuOH (90%). **f:** 9b, KOH (3 eq.), THF-H_2O (95%). **g:** H_3O^+, AG50W resin chrom'y, 2N NH_4OH (84%). **h:** 14 or 15, chrom'y.; KOH-H_2O. **I:** $PhCH_2OCOCl$, Et_3N, THF; **or** (*t*-$BuOCO)_2O$, Et_3N, THF (~80%). **J:** DCHA, Et_2O (fractional xtal'n of 18a). **k:** 1 N HCl-HOAc 0.5 hr., r.t. (72%). **l:** bis(trimethylsilyl)acetamide (3 eq.), CH_3CH, 18 hr., r.t. (quant.). **m:** *N*-(benzyloxycarbonyloxy)-succinimide **or** [2-(*t*-butoxycarbonyloxyimino)phenylacetonitrile]. **n:** N-methyl-N-(*t*-butyldimethylsilyl)trifluoroacetamide (21).

In contrast to its phenyl counterpart,[6,8] reduction of **8** according to reported conditions[10] again produced erratic low yields of **9a**.[17] This might be attributable to more facile oligomerization of the amino ester **9a** during reaction and isolation, but hydrolysis of **8** to **10**[18] afforded access to more useful derivatives. Attempted DIBAL reduction of the *t*-butyl amide **11** according to conditions described in the phenyl series[19] led only to intractable mixtures. The *t*-butyl ester **12**,[20] however, led to an 80% yield of **9b** (isolated as 2HCl salt)[21] under the standard Zn-Cu/AcOH reduction conditions.[10] Although formation of the HCl salt of **9b** before work-up prevented subsequent decomposition, this did not

work well in the case of the ethyl ester **9a**. All attempts to protect the γ-hydroxy and α-amino functionalities of **9b**•2HCl proved futile, leading only to the lactones **13**, which were isolated as the benzyl or *t*-butyl carbamates **14** or **15**.[22]

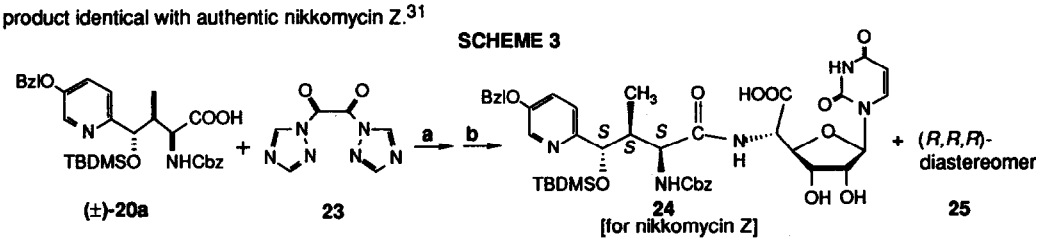
The *t*-butyl ester **9b** was remarkably easy to hydrolyze to **16**.[23,24] The α-amino group of **16** could easily be protected as *N*-CBZ or *N*-BOC derivatives **17** or **18**, reasonably stable as dicyclohexylamine (DCHA) salts. Fortuitously, (*R**,*R**,*R**)-**18**•DCHA selectively crystallized from the diastereomer mixture in Et_2O offering an alternate separation method. Not surprisingly, formation of the active esters of **17** or **18** led only to the lactones **14** or **15**. But all attempts to further protect the free hydroxyl group in either **17** or **18** also failed, the most frequent products being the lactones **14** or **15**. It appears that the presence of bulky *N*-protecting groups in **17** and **18** provide significant steric constraint about the γ-hydroxyl for intermolecular reactions. An added factor for this reduced reactivity could well be attributable to coordination effects of the proximate pyridyl nitrogen.

In an alternative approach, persilylation of **16a**, followed by *in situ* amino protection as CBZ or BOC was then tried. It was reasoned that even if *N*-silylation took place before *O*-silylation, an intramolecular *N*→*O* silyl transfer would amount to net *O*-silylation; and the silyl ester would hydrolyse during work-up. This concept was successfully carried out to provide the suitably protected *O*-trimethylsilyl acids **19a** and **19b**. These derivatives however, proved only marginally suitable as they were unstable to storage, leading to the lactones **14** and **15**.[25] Subsequently, a *t*-butyldimethyl silylation protocol gave **20a**[26] and **20b** which were perfectly stable to work-up and storage. It is important to note that use of highly reactive *N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide (**21**) was necessary, possibly acting *via* the intermediate **22**. Later coupling experiments showed that **20a** was much preferred over **20b** based on reduced yields with the latter, presumably due to steric reasons.

The difficulties encountered in coupling of protected and relatively unhindered amino acids with **2** were reportedly overcome by use of DMSO as solvent and diisopropylethylamine as base.[27] This expedient alone proved ineffective in attempts to couple **20a** with **2** by either the mixed anhydride or DCC/HOSu active ester method (maximum yield 14%); the activation step alone required over 4 days at 20°C. What appeared to be severe steric hindrance required that the activated acyl group had to be much more electrophilic, and that the activating reagent had to be also highly reactive.

Carbonylditriazole[28] and oxalylditriazole (**23**),[29] two virtually non-basic reagents seemed to meet the above criteria. The latter virtually overlooked reagent **23** was first described as a potent carboxylic acid activator for esterification,[29] and subsequently as a condensing agent to prepare amides, including hindered dipeptides.[30] Indeed, activation of (*R,*R,*R**)-**20a** with **23**, followed by coupling with **2** (Scheme 3), afforded **24** as one of the two diastereomers in 50-55% total yield; the conventional methods had failed to exceed 15% yields. Finally, the one-pot deprotection of **24** was accomplished using Pd-black/HCOOH-MeOH-H_2O (90:9:1)/20°C/1-2 hr. to provide a single product identical with authentic nikkomycin Z.[31]

SCHEME 3



Reagents. a: CH_3CN, 20°C, 3 hr. **b:** 2, N-methylmorpholine, DMSO, 20°C, 48 hr.

Since uracil polyoxin C (**2**) has been obtained by total synthesis,[32] this constitutes formally the first total synthesis of nikkomycin Z. The synthesis of a (storable) suitably protected (5'-hydroxy)nikkomycin E derivative **20a**, and coupling

procedure described above are capable of providing any of the several stereoisomers of nikkomycin Z with appropriate modification. This work uses and augments the available strategies pertaining to resolution[8,9] and stereoselective synthesis[11] for **1b**. We shall detail on some of these other aspects in forthcoming communications.

REFERENCES AND NOTES

‡ Presented in part at the 204th American Chemical Society Meeting, Washington, D.C., August 1992

1. Mueller, H; Eurter, R.; Zähner, H.; Rast D.M. *Arch. Microbiol.* **1981**, *130*, 195-197.
2. Becker, J.M.; Marcus, S.; Tullock, J.; Miller, D.; Krainer, E.; Khare, R.K.; Naider, F. *J. Infect. Diseases* **1988**, *157*, 212-214.
3. Pappagianis, D.; Zimmer, B.; Hector, R.V. *Abstr. 28th Intersci. Conf. on Antimicrob. Agents &Chemother.* **1988**, 287, abs. 1004.
4. König, W.A.; Hahn, H.; Rathmann, R.; Hass, W.; Keckeisen, A.; Hagenmaier, H.; Bormann, C.; Dehler, W.; Kurth, R.; Zähner, H. *Justus Liebigs Ann. Chem.* **1986**, 407-421, and prior references cited therein.
5. König, W.A.; Pfaff, K-P.; Batsch, H-H.; Schmalle, H.; Hagenmaier, H. *Justus Liebigs Ann. Chem.* **1980**, 1728-1735.
6. König, W.A.; Hass, W.; Dehler, W.; Fiedler, H-P.; Zähner, H. *Justus Liebigs Ann. Chem.* **1980**, 622-628.
7. Since **1b** is an hydroxylated analog of known nikkomycin E, we refer to it here trivially as (5'-hydroxy)nikkomycin E.
8. Zimmermann, G.; Hass, W.; Faasch, H.; Schmalle, H.; König, W.A. *Justus Liebigs Ann. Chem.* **1985**, 2165-2177.
9. Hahn, H.; Heitsch, H.; Rathmann, R.; Zimmermann, G.; Bormann, C.; Zähner, H. König, W.A. *Justus Liebigs Ann. Chem.* **1987**, 803-807.
10. Hass, W.; König W.A. *Justus Liebigs Ann. Chem.* **1982**, 1615-1622.
11. Barrett, A.G.M.; Lebold, S. *J. Org. Chem.* **1991**, 4875-4882; and references therein to other groups' efforts.
12. Previously described and elaborated in a more general context: (a) Drefahl, G.; Horhold, H.H. *Chem. Ber.* **1964**, *97*, 159: (b) Jager, V.; Bu8, V..; Schwab, W. *Tetrahedron Lett.* **1978**, 3133.
13. When **3a** was first benzylated, the mono-lithiation/alkylation attempt gave only tars.
14. New compounds were characterized by mass spectrum, elemental analysis for crystalline compounds only, and proton NMR. Yields refer to isolated products. Selected spectral data are given:
    For **4**: H^1 NMR (acetone-d_6+D_2O) δ 8.1 (m,1), 7.2 (m, 2), 4.1 (m, 1), 2.76 (d, 2), 1.10 (d, 3). (b) For **5**: H^1 NMR ($CDCl_3$+D_2O) δ 8.22 (d, 1), 7.38 (s, 5), 7.4-7.0 (m, 2), 5.00 (s, 2), 4.19 (m, 1), 2.8 (m, 2), 1.27 (d, 3).
15. Other protecting groups were tried with noticeably inferior overall results.
16. For **8**: A mixture of 40 g (0.18 mole) **6**, 2.5 L Et_2O, and 107 g (0.69 mole) **7** was vigorously stirred in a 5-L Morton flask at r.t. A solution of 37 g (0.35 mole) Na_2CO_3 in 440 mL water was added via a constant rate addition funnel (a mechanical syringe pump was more suitable on smaller scale) at the rate of 12-14mmol Na_2CO_3/hr. per mmol **6**. After complete addition (70-78 hr.), the ethereal layer was separated, washed with saturated NaCl, and dried over anhydrous $MgSO_4$. The mixture was filtered, evaporated, and the residue chromatographed on silica gel to give 45 g **8** (74%), $C_{19}H_{20}N_2O_4$ (340.38): MS (FAB) 341 ($M+H^+$, 100%); H^1 NMR ($CDCl_3$) δ 8.37 (d, 1), 7.36 (s, 5), 7.2 (m, 2), 5.36 (d, 1), 5.09 (s, 2), 4.32 (q, 2), 3.79 (m-5, 1), 1.6-1.2 (m-4, 6).
17. A modified procedure based on analogous diastereomeric amides rather than **8** was published[8] subsequently.
18. For **10**: mp 137-8°C (dec); H^1 NMR (DMSO-d_6) δ 8.42 (m, 1), 7.4 (m, 7), 5.39 (d, 1), 5.22 (s, 2), 3.80 (p, 1), 1.38 (d, 3).
19. Banks, B.; Barrett, A.G.M.; Russell, M.; Williams, D. *J. Chem. Soc. Chem. Commun.* **1983**, 873-875.
20. For **12**: To a solution of 29 g (93 mmol) **10** in 250 mL DMF at 0°C was added 16 g (98 mmol) Im_2CO, and the mixture was stirred 1 hr. at r.t. After cooling to 0°C, 10 mL *t*-BuOH and 10.4 g (93 mmol) *t*-BuOK were added, and the mixture stirred 1 hr. at r.t. The solution was diluted with aq. $NaHCO_3$ and extracted with EtOAc. The extract was dried and evaporated, and the residue was chromatographed (EtOAc-hexane) to afford 22 g **12** (64%). A portion was crystallized from Et_2O-hexane: $C_{21}H_{24}N_2O_4$ (368.44); H^1 NMR ($CDCl_3$) δ 8.32 (d, 1), 7.35 (s, 5), 7.23 (m, 2), 5.24 (d, 1), 5.07 (s, 2), 3.70 (m, 1), 1.5 (s, 9), 1.42 (d, 3).
21. For **9b**: A solution of 24.5 g (58 mmol) **12** in 1.1 L HOAc was reduced with 330 g freshly prepared Zn-Cu couple over 7 hr.[10] The mixture was filtered, and 2.3 eq. of aq. HCl was added. Evaporation, and trituration of the residue with Et_2O afforded 32 g of a mixture of **9b** as a dihydrochloride together with metal acetate salts. This could not be purified without loss but could be stored.
22. The lactones proved useful for chromatographic separation of the pairs of racemic (*R*,R*,R**) and (*R*,R*,S**) diastereomers.
23. Isolation from the acidified hydrosylate was best done by passage through a Dowex 50W X8 ion exchange resin, and elution with 0.5-1 N aq. NH_4OH. This served to remove any remaining Cu and Zn ion, which can severely alter results of subsequent protection steps. Lactonization was negligible during the workup/isolation periods.
24. For **16a**: H^1 NMR (DMSO-d_6) δ 8.37 (m, 1), 7.5-7.25 (m, 8), 5.15 (s, 2), 4.43 (m, 1), 3.38 (m, 1), 3.00 (m, 1), 1.03 (d, 1/2 of 3H), 0.71 (d, 1/2 of 3H).
25. We surmise that some γ-*O* → carboxyl-*O* silyl migration occurs in **19**, and the resultant ester quickly lactonizes.
26. For **20a**: A mixture of 100 mg **16a** 2 mL anhydrous CH_3CN, and 155μL (2.1 eq) (TBDMS)N(Me)$COCF_3$ (**21**)[33] was stirred 24-42 hr. at r.t. Then 83 mg N-(benzyloxycarbonyl)succinimide was added and stirred 24-48 hr. at r.t. Cold 5% KH_2PO_4 was added, extracted with EtOAc, dried over $NaSO_4$, and evaporated . The residue was dissolved in THF, treated with 1 mL 1:1 MeOH-H_2O and 50 mg K_2CO_3, and stirred 0.5 hr. at r.t. to hydrolyze TBDMS esters. Extraction and evaporation afforded 165 mg **20a** as a gum containing a small amount of lactones, but otherwise cleanly 2 isomers by TLC, and used without further purification: $C_{31}H_{40}N_2O_6Si$ (564.76) ms (FAB) 565 ($M+H^+$, 100%); H^1 NMR ($CDCl_3$) δ 8.42 (s, 1/2 of 1H), 8.25 (d, 1/2 of 1H), 7.6-7.3 (m, 12), 5.9 (d, 1/2 of NH), 5.6(d, 1/2 of NH), 5.18 (s, 2), 5.12 (m, 2), 4.82 (m,1/2), 4.11 (t, 1/2), 2.4-2.2 (m, 1), 0.93 (s, 9), 0.85 (d, 3/2), +0.12 to -0.4 (multiple peaks, 3/2 + 6).
27. Krainer, E.; Becker, J.M.; Naider, F. *J. Med. Chem.* **1991**, *34*, 174-180.
28. Walter, W.; Radke, M. *Justus Liebigs Ann. Chem.* **1979**, 1756-1767.
29. Murata, S. *Bull. Chem. Soc. Japan* **1984**, *57*, 3597-3598.
30. Kitagawa, T.; Kuroda, H.; Sasaki, H.; Kawasaki, K. *Chem. Pharm. Bull. Japan* **1987**, *35*, 4294-4301.
31. Purchased from Calbiochem Corp., San Diego, CA.
32. Vogel, P.; Auberson, Y. *Tetrahedron* **1990**, *46*, 7019-7032, and references cited therein.
33. Mawhinney, T.P.; Madson, M.A. *J. Org. Chem.* **1982**, *47*, 3336.

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