Synthetic Study of Kedarcidin Chromophore: Revised Structure

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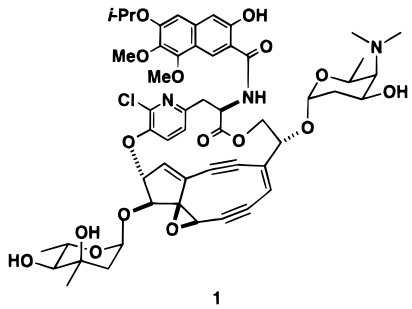
Kedarcidin is a new chromoprotein antitumor antibiotic family consisting of a carrier apoprotein and a cytotoxic nine-membered enediyne chromophore.[1] Although the naked chromophore is highly unstable, its structure including the absolute configuration was described as **1** in 1993.[2] Because of its unusual structure, extremely potent antitumor activity, and high degree of sequence specificity in DNA cleavage,[3] we attempted to synthesize this chromophore. The core moiety of *ent*-**1** has recently been stereoselectively synthesized.[4,5] In this communication, we describe the synthesis of the chloroazatyrosyl naphthoamide fragment **2** of *ent*-**1**. We demonstrate that the fragment degraded from the chromophore is not an α-amino acid derivative but *β*-amino ester **3** and that the structure of kedarcidin chromophore should be revised to be **27**.

An efficient route to the polysubstituted naphthoic acid **11** was first established (Scheme 1). Methyl gallate (**4**) was regioselectively *O*-alkylated using isopropyl iodide after acetalization of the vicinal dihydroxy group in 72% overall yield.[6] Regioselective bromination[7] of phenol **5** followed by methylation of the two phenolic hydroxy groups yielded appropriately functionalized benzoate **6**. Homologation of ester **6** via Wolff rearrangement gave phenylacetate **7**. The Heck reaction of **7** with *tert*-butyl acrylate afforded the α,*β*-unsaturated ester **8**.[8] After methyl ester **8** was converted to acid chloride **9**, cyclization to form a naphthalene ring was examined by the intramolecular Friedel−Crafts reaction of **9** with $AlCl_3$. However, the yield of **12** from **8** was low (0−36%) after esterification with MeOH, EDC·HCl, and DMAP. Another route via an electrocyclic reaction of the ketene intermediate proceeded smoothly.[9,10]



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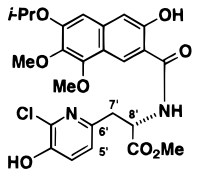

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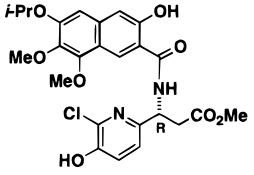

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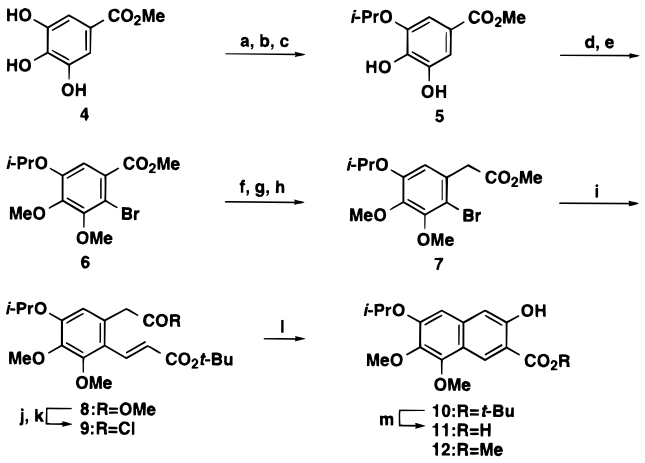
Scheme 1[a]



[a] Reagents and conditions: (a) Ph_2CCl_2,, 175 °C. (b) *i*-PrI, K_2CO_3, acetone, 50 °C. (c) AcOH, H_2O, reflux, 72% (three steps). (d) NBS, THF, room temperature (rt). (e) MeI, K_2CO_3, acetone, 50 °C, 86% (two steps). (f) NaOH, MeOH, H_2O, 60 °C. (g) $(COCl)_2$, $PhCH_3$. (h) CH_2N_2, Et_3N, Et_2O; then $PhCO_2Ag$, Et_3N, MeOH, 75% (three steps). (i) $CH_2{=}CHCO_2t$-Bu, $Pd(OAc)_2$, $(p\text{-tol})_3P$, Et_3N, 100 °C, 90%. (j) $Ba(OH)_2{\cdot}8H_2O$, *t*-BuOH. (k) $(COCl)_2$, CH_2Cl_2. (l) *i*-Pr_2NEt, $PhCH_3$, rt, 62% (three steps). (m) CF_3CO_2H, 99%.

Treating the acid chloride **9** with *i*-Pr_2NEt (1 equiv) in toluene at room temperature gave *tert*-butyl naphthoate **10** in 62% yield, which was readily converted to naphthoic acid **11**.

The chloroazatyrosine derivative[11] **16** was efficiently synthesized from commercially available 2-chloro-3-hydroxypyridine (**13**) (Scheme 2). The palladium-catalyzed cross-coupling reaction of iodopyridine **14** and the alkylzinc compound **15** prepared from L-serine[12,13] followed Burke's procedure.[14] The TBS group was removed to form **16** using a weakly acidic aqueous mixture of HF−NaF. Deprotection of the Boc group of **16** and subsequent coupling with **11** gave α-chloroazatyrosyl

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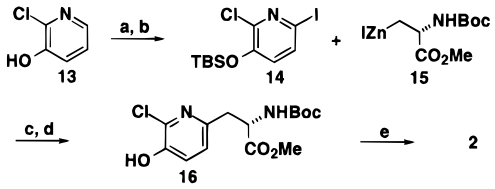
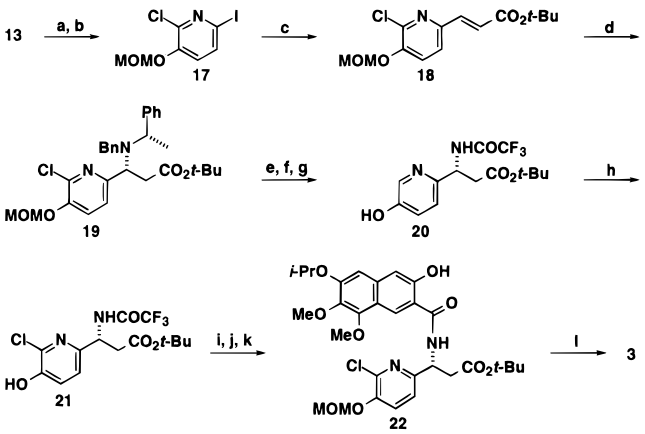
Scheme 2[a]



[a] Reagents and conditions: (a) *t*-BuOCl, NaI, MeCN, H_2O. (b) TBSCl, imidazole, DMF, 71% (two steps). (c) $PdCl_2(PPh)_2$, *N*,*N*-dimethylacetamide, THF, 60 °C. (d) 0.1 M HF, 0.1 M NaF, pH 5, THF, 73% (two steps). (e) 4 N HCl in 1,4-dioxane; then **11**, EDC·HCl, HOBt, Et_3N, DMF, 82%.

Scheme 3[a]

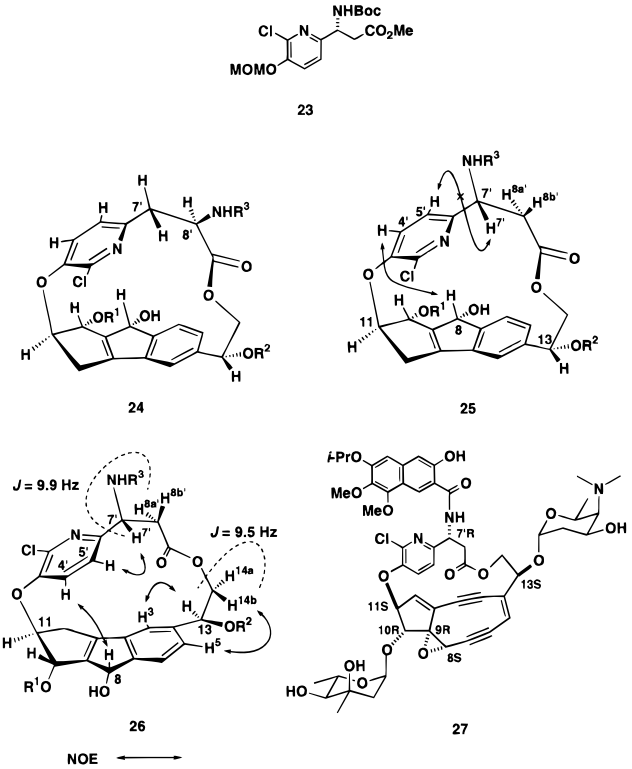


[a] Reagents and conditions: (a) *t*-BuOCl, NaI, MeCN, H_2O. (b) MOMCl, K_2CO_3, acetone, 50 °C, 75% (two steps). (c) $CH_2{=}CHCO_2$-*t*-Bu, $Pd(OAc)_2$, Et_3N, DMF, 60 °C, 90%. (d) (*S*)-(−)-benzyl-α-methylbenzylamine, BuLi, THF, −80 °C, 1 h; then saturated NH_4Cl (aq), 83%. (e) $Pd(OH)_2/C$, H_2, MeOH, H_2O, AcOH. (f) CF_3CO_2Et, Et_3N, MeOH, 88% (two steps). (g) TsOH, *t*-BuOH, 55 °C, 75%. (h) NCS, DMF, 70 °C, 82%. (i) MOMCl, K_2CO_3, acetone, (j) K_2CO_3, MeOH, H_2O. (k) **11**, EDC·HCl, HOBt, CH_2Cl_2, 57% (three steps). (l) CF_3CO_2H; then MeOH, EDC·HCl, DMAP, CH_2Cl_2, 54%.

naphthoamide **2**. The ^1H and ^{13}C NMR spectra as well as the CD spectrum of synthetic **2**, however, were not identical with the reported data[2b] (see the Supporting Information).

We assumed that the chloroazatyrosine moiety of kedarcidin would be the corresponding β-amino acid because of marked differences in the NMR chemical shifts at C5′, 6′, 7′, and 8′ of **2**. The optically active β-chloroazatyrosine derivative **3** was synthesized via an enantio-face-selective conjugate addition of Davies' chiral lithium amide to the α,β-unsaturated ester **18** (Scheme 3). We predicted the *R* configuration of the major β-amino ester **19** (93:7) on the basis of Davies' experimental results.[15] Subsequent selective hydrogenolysis of the benzyl and phenethyl groups on the amine **19** proceeded under various reduction conditions without destroying β-amino ester functionality,[15] but the chloride was always partially or completely removed. Therefore, after the amino group was protected as a trifluoroacetoamide, phenol **20** was regioselectively chlorinated with NCS. Protection of the phenolic hydroxy group of **21** was indispensable prior to the alkaline hydrolysis of trifluoroacetamide, and the resulting amine was coupled with **11** to give naphthoamide **22**. Acidic hydrolysis of both the MOM ether and *tert*-butyl ester groups followed by esterification yielded (*R*)-β-chloroazatyrosyl naphthoamide **3**. The spectral data (^1H and ^{13}C NMR, UV, and CD spectra as well as optical rotation) of **3** were identical with those reported for the fragment[2b] degraded from kedarcidin chromophore (see the Supporting Information). The *R* stereochemistry was confirmed by the synthesis of **3** from **23**,[16] the *R* configuration of which was defined using a modified Mosher method.[17]

Therefore, we reexamined the entire structure of the chromophore. The structures of the sugar moieties were unequivocally determined by comparison with an authentic sample[2b] and by X-ray crystallographic analysis.[18] Close inspection of the reported NMR data for stable sodium borohydride reduction product assigned as **24**[2b] indicated that ambiguity remained only in the stereostructure of the core. There are two possible diastereomeric structures, **25** and **26**, with a (*R*)-β-chloroazatyrosyl naphthoamide bridge, in which free rotation of the azatyrosine unit is restricted.[2b] Only **26** is fully consistent with the reported NOEs and $^1H-^1H$ coupling constants:[2b] four key NOEs between H4′ and H8, H5′ and H7′, H3 and H13, and H5 and H14b as well as two large coupling constants, $J = 9.9$ and 9.5 Hz for H7′−H8a′ and for H13−H14b, respectively. The NOE between H5′ and H7′ ruled out the possibility of **25**, which has the same core configuration as **24**. Thus, the complete structure of the kedarcidin chromophore should be revised to that of **27**.



The present results should accelerate studies not only of kedarcidin chromophore synthesis but also of interactions with biopolymers at a molecular level.[3]

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Supporting Information Available: Spectral data of compounds **2**, **3**, **6**−**8**, **10**−**12**, **14**, and **16**−**23** and synthetic scheme for **23** (21 pages). See any current masthead page for ordering and Internet access instructions.

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(16) Both enantiomers were synthesized by a conjugate addition of hydroxylamine to the α,β-unsaturated carboxylic acid corresponding to **18** followed by the protection of the resulting amino acid (Boc_2O, NaOH, 1,4-dioxane, H_2O; MeI, $KHCO_3$, DMF) and subsequent optical resolution (HPLC with CHIRALCEL OD).

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