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Efficient total synthesis of (+)-negamycin, a potential chemotherapeutic agent for genetic diseases†

Yoshio Hayashi,*[ab] Thomas Regnier,[a] Shigenobu Nishiguchi,[a] Magne O. Sydnes,[a] Daisuke Hashimoto,[c] Junya Hasegawa,[c] Takahiro Katoh,[c] Tetsuya Kajimoto,[c] Masataka Shiozuka,[d] Ryoichi Matsuda,[d] Manabu Node[c] and Yoshiaki Kiso*[a]

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Herein, we describe an efficient strategy for the total synthesis of (+)-negamycin using commercially available achiral *N*-Boc-2-aminoacetaldehyde as starting material with 42% overall yield for a limited number of steps.

(+)-Negamycin (**1**, Scheme 1), an unusual antibiotic containing a hydrazine peptide bond was isolated for the first time by Umezawa *et al*. in 1970 from culture filtrates of three strains related to *Streptomyces purperfuscus*. This natural product exhibits very low acute toxicity and strong inhibitory activity against multiple drug-resistant enteric Gram-negative bacteria including *Pseudomonas aerginosa*.[1] (+)-**1**'s anti-microbial activity is derived from a genetic miscoding on bacterial ribosomal systems, and thereby leading to a specific inhibition of protein biosynthesis.[2] Because this miscoding causes read-through of termination signals, considerable attention is focused on (+)-**1** as a potential therapeutic agent against genetic diseases. Indeed, the aminoglycoside antibiotic gentamicin and the less toxic negamycin both restore dystrophin expression in skeletal and cardiac muscles of mdx mice, an animal model of Duchenne muscular dystrophy (DMD) with a nonsense mutation in the dystrophin gene.[3] Therefore, an efficient shortened synthetic route of (+)-**1** and its derivatives appears significant to develop promising new therapeutic candidates for DMD and other diseases caused by nonsense mutations. The first total synthesis of (+)-**1** from D-galacturonic acid was reported in 1972 and confirmed the assigned structure of the natural product.[4] Over three decades, numerous total syntheses have been reported on both racemic and optically active (+)-**1** but with moderate overall yield.[5]

Our fast and efficient route consists first on an asymmetric allylboration of *N*-Boc-glycinal **5** using the established Brown's procedure for preparation of chiral allylic alcohols[6] that led to a corresponding chiral intermediate (Scheme 1). This resulting chiral amino alcohol was directly engaged without further purification to form the target oxazolidine **4** by treatment with 2,2-dimethoxypropane (DMP) in the presence of boron trifluoride diethyl ether complex ($BF_3{\cdot}Et_2O$) in acetone. As a result, **4** was generated in high yield after purification by silica gel column chromatography (90%). To prepare the key intermediate **3**, a cross-metathesis (CM) reaction between **4** and *tert*-butyl acrylate **6** was investigated. Although the efficiency of ruthenium-based catalysts for ring-opening metathesis polymerization (ROMP) and ring-closing metathesis (RCM) is now well established, most alkene CM variants have fewer successful applications because of the multiple possible side reactions that cause relatively low synthetic yields.[7] Because our substrates are categorized as rapid and slow homodimerizable compounds according to Grubbs *et al*.'s empirical model for predicting the outcome of CM reactions,[8] we screened different reaction conditions to avoid forming unwanted dimers and selectively provide the target compound **3** by varying catalysts (Grubbs first [Ru-**I**] and second-generation [Ru-**II**] catalysts), amount of reactant (1 or 5 equiv. of **6**), duration of reaction as well as heating method (conventional or microwave-assisted heating).

As detailed in the ESI,† the conversion and chemoselectivity enhancements are definitely more pronounced for [Ru]-**II** than [Ru]-**I**. Furthermore, we observed that microwave irradiation drastically shortened the CM reaction time by 20-fold. As it pertains to microwave-assisted synthesis, this acceleration is commonly attributed to the very high local temperatures and the ease to which microwave irradiation reaches such conditions but the scientific community is still divided in opinion on the involvement of a specific non-thermal effect induced by the dielectric heating produced using microwaves.[9] Interestingly, although our observations provide a new example for the thermal effect, the involvement of such "specific effect" is neither confirmed nor disproved in these reaction conditions. Bargiggia *et al*. arrived to similar conclusions while studying CM reactions[10] and Garbaccia *et al*. have described similar observations for RCM reactions in 2003.[11] As a result, the desired product **3** was isolated with 83% yield. NOE experiments revealed that the stereochemistry of the olefin moiety in **3** was an *E* configuration ($J_{\text{vinylic protons}}$ = 15.7 Hz). Thus, the desired chiral intermediate **3** was obtained with 75% yield after two steps from achiral *N*-Boc-glycinal **5**.

With intermediate **3** in hand, our focus shifted toward the asymmetric Michael addition reaction. Recently, Node *et al*.

[a] *Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Kyoto 607-8412, Japan. E-mail: yhayashi@ps.toyaku.ac.jp, kiso@mb.kyoto-phu.ac.jp; Fax: +81-75-595-4787; Tel: +81-75-595-4636*
[b] *Department of Medicinal Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo 192-0392, Japan*
[c] *Department of Pharmaceutical Manufacturing Chemistry, Kyoto Pharmaceutical University, Kyoto 607-8412, Japan*
[d] *Department of Life Science, University of Tokyo, Tokyo 153-8902, Japan*

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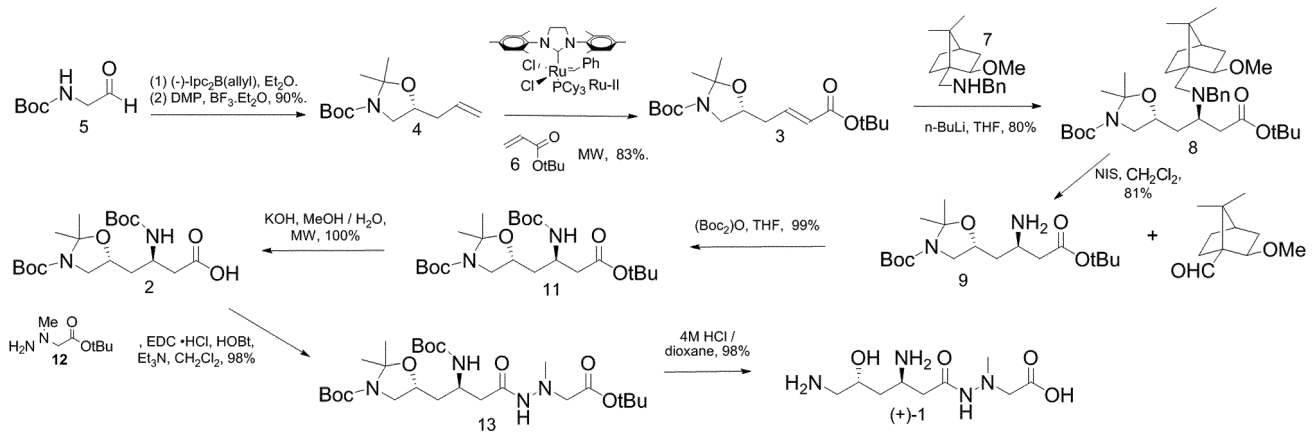

Scheme 1 Total synthesis of (+)-negamycin.

reported a highly stereoselective asymmetric Michael addition toward *tert*-butyl α,β-unsaturated carbonyl compounds using chiral amine **7**.[12] This approach, when applied to the α,β-unsaturated *tert*-butyl ester **3**, allowed the introduction of the amine moiety with an excellent enantiomeric excess.[13] The chiral reagent **7** was prepared from the corresponding keto-pinic acid[12] and reacted with **3** in the presence of *n*-BuLi in THF at −78 °C to afford compound **8** as a single diastereomer (de >99%) in 80% yield after purification. Removal of both benzyl and 2-methoxybornyl protecting groups located on the same amine moiety could be achieved efficiently using 4 equiv. of *N*-iodosuccinimide (NIS) in dichloromethane to obtain free amino compound **9** in 81% yield. This deprotection proceeded by oxidation with NIS to imine and subsequent spontaneous hydrolysis to afford *tert*-butyl esters of β-amino acids and 2-methoxy-D-bornylaldehyde.[12] No epimerization was observed during this reaction. Furthermore, one of the advantages of the protocol is that the initial chiral inducer **7** can be easily regenerated from 2-methoxy-D-bornaldehyde, generated during the cleavage by reductive amination, using benzylamine in the presence of sodium cyanoborohydride (data not shown).

The last part of the synthesis of (+)-**1** consisted of introducing a hydrazine unit, prior to a final deprotection. A Boc-protection of **9** using standard procedures was first quantitatively performed to afford *N*-protected *tert*-butyl ester **11**, that was then efficiently converted to acid **2** by a microwave-assisted saponification with 2M KOH in MeOH, and coupling with hydrazine unit **12** was then performed using the classical EDC·HCl–HOBt method. The synthesis of hydrazine **12** was achieved by reacting *N*-methyl hydrazine with *tert*-butyl bromoacetate with 40% yield after purification. Deprotection of compound **13** and purification by ion exchange chromatography on Amberlite CG50 (NH_4^+ form) afforded the target compound (+)-**1** in 98% yield, $[\alpha]_D^{25.2}$ +2.4° (*c* 0.36, H_2O), lit. $[\alpha]_D^{29.0}$ + 2.5° (*c* 2.00, H_2O). The final compound was fully characterized and compared with the published data for the natural product to confirm the success of this new total synthesis of (+)-**1** (*e.g.* ^{1}H NMR data for natural and synthesized (+)-**1**, available in ESI†). Furthermore, the *in vivo* read-through activity of termination codons during protein biosynthesis[3] of the synthesized (+)-**1** in mice was very similar to that of the native (+)-**1** (data not shown). Further derivatization of the **1** structure using the above synthetic methodology will contribute to a better understanding of the structure–activity relationship of **1** and the development of more potent compounds with efficient read-through activity. Studies in this regard are currently in progress and details pertaining to the biological activity will soon be published elsewhere.

In conclusion, the proposed synthetic route for the total synthesis of optically active (+)-negamycin starting from *N*-Boc-glycinal **5**, led to the desired product with a total yield of 42% over only eight steps. To our knowledge, this study represents the most efficient strategy to prepare (+)-**1**. Current efforts with this new synthetic approach are now expanding into medicinal chemistry to discover new drug candidates with potent read-through activity for Duchenne muscular dystrophy. The chemical biology of negamycin is also now being investigated to better understand its read-through mechanism.

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