Total Synthesis of (+)-Neocarzinostatin Chromophore

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Total Synthesis of (+)-Neocarzinostatin Chromophore

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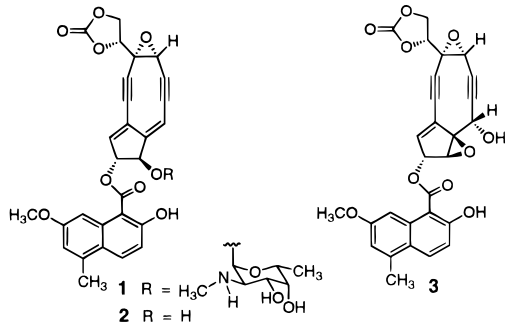
The chromoprotein enediyne antibiotics are characterized by the high reactivity of their "enediyne" chromophore components, a feature that greatly complicates the development of synthetic routes to these agents.[1] To date, no chromoprotein chromophore has been synthesized, although notable achievements in this area have been reported.[2,3] We describe below a total synthesis of (+)-neocarzinostatin chromophore, the first of the enediyne antibiotics to be characterized.[4] The route employed makes use of an atypical protocol for the introduction of a 2-amino sugar that may find broader applicability in the synthesis of other aminoglycosides.



In prior work, we described an enantioselective synthesis of (+)-neocarzinostatin chromophore aglycone ((+)-**2**)[5] involving as the final step a novel reductive deoxygenation of the epoxy alcohol **3** using triphenylphosphine, iodine, and imidazole.[6] This transformation has since been markedly improved by the introduction of a low-temperature (−10 °C) methanol quench and by conducting extractive and chromatographic isolation procedures at 4 °C under an inert atmosphere. With these modifications, the efficiency of the transformation has more than doubled, such that (+)-**2** is obtained reproducibly in 65−72% yield after chromatographic purification. The aglycone (**2**) was prepared with the objective of implementing a final-stage glycosylation reaction for the synthesis of **1** using a suitably protected glycosyl donor. Although **1** readily decomposes when separated from its binding protein, mildly acidic conditions are tolerated. For this reason these conditions were chosen for the proposed coupling and deprotection steps.[7] In this regard, the Schmidt trichloroacetimidate method was felt to be an ideal procedure for the glycosylation reaction in light of its versatility and, more importantly, for the mildly acidic conditions used in the coupling reaction.[8] Reports of the use of the Schmidt methodology for the synthesis of 2-amino sugars have primarily described the use of a 2-azido substituent as a latent amino functionality.[8,9] This was felt not to be a viable option in the present case because of the incompatibility of the chromophore with conditions for the reduction of the azide and as well for the monomethylation of the resultant amine. We therefore chose to employ a glycosyl donor containing a preexisting 2-methylamino group and focused on the selection of protective groups for this functionality and for the two hydroxyl groups. A goal was to remove all protective groups in a single operation after glycosylation, an approach that was dictated by the unstable nature of the chromophore.

After extensive experimentation, we determined that the C-3 and C-4 hydroxyl groups were best masked as triethylsilyl (TES) ethers,[10] but we were unable to find a suitable means to protect the *N*-methylamino group. Carbamates such as 2-(3,5-dimethoxyphenyl)propyloxycarbonyl led to oxazolidinone formation during the coupling reaction, while *t*-Boc and 2-(trimethylsilyl)ethoxycarbonyl (TEOC) groups were found to be too robust to be cleaved under conditions that **1** would survive. Bulky groups, such as *N*-bis(4-methoxyphenyl)methyl, tended to block attack of the glycosyl acceptor from the α-face, so that the undesired *β*-anomeric products predominated. Eventually, we were led to question whether an *N*-methylamino protective group was necessary at all. Despite the obvious appeal of such a strategy, we were unaware of any examples in which the trichloroacetimidate method had been conducted in the presence of a free amino group and had concerns about the viability of such a proposal given that the coupling is promoted by substoichiometric Br*ϕ*nsted or Lewis acids that might be inactivated by reaction with the amine.[11,12]

To explore this possibility, the glycosyl donor **4**[13] containing a free *N*-methylamino group was synthesized in 10 steps from tri-*O*-acetyl-D-galactal (see Supporting Information). Studies of

(1) Reviews: (a) Smith, A. L.; Nicolaou, K. C. *J. Med. Chem*. **1996**, *39*, 2103. (b) Grissom, J. W.; Gunawardena, G. U.; Klingberg, D.; Huang, D. *Tetrahedron* **1996**, *52*, 6453.

(2) (a) Myers, A. G.; Harrington, P. M.; Kuo, E. Y. *J. Am. Chem. Soc*. **1991**, *113*, 694. (b) Myers, A. G.; Harrington, P. M.; Kwon, B.-M. *J. Am. Chem. Soc*. **1992**, *114*, 1086.

(3) For leading references to other synthetic studies of **1** and related model systems, see: (a) Wender, P. A.; Harmata, M.; Jeffrey, D.; Mukai, C.; Suffert, J. *Tetrahedron Lett*. **1988**, *29*, 909. (b) Hirama, M.; Fujiwara, K.; Shigematu, K.; Fukazawa, Y. *J. Am. Chem. Soc*. **1989**, *111*, 4120. (c) Suffert, J. *Tetrahedron Lett*. **1990**, *31*, 7437. (d) Doi, T.; Takahashi, T. *J. Org. Chem*. **1991**, *56*, 3456. (e) Hirama, M.; Gomibuchi, T.; Fujiwara, K. *J. Am. Chem. Soc*. **1991**, *113*, 9851. (f) Nakatani, K.; Arai, K.; Terashima, S. *Tetrahedron* **1993**, *49*, 1901. (g) Matsumoto, Y.; Kuwatani, Y.; Ueda, I. *Tetrahedron Lett*. **1995**, *36*, 3197. (h) Toshima, K.; Ohta, K.; Yanagawa, K.; Kano, T.; Nakata, M.; Kinoshita, M.; Matsumura, S. *J. Am. Chem. Soc*. **1995**, *117*, 10825. (i) Magnus, P.; Carter, R.; Davies, M.; Elliott, J.; Pitterna, T. *Tetrahedron* **1996**, *52*, 6283. (j) Caddick, S.; Delisser, V. M. *Tetrahedron Lett*. **1997**, *38*, 2355. (k) Ferri, F.; Brückner, R. *Liebigs Ann*. **1997**, 961.

(4) Isolation of **1**: (a) Napier, M. A.; Holmquist, B.; Strydom, D. J.; Goldberg, I. H. *Biochem. Biophys. Res. Commun*. **1979**, *89*, 635. (b) Koide, Y.; Ishii, F.; Hasuda, K.; Koyama, Y.; Edo, K.; Katamine, S.; Kitame, F.; Ishida, N. *J. Antibiot*. **1980**, *33*, 342. Chromophore structure: (c) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett*. **1985**, *26*, 331. Carbohydrate stereochemistry: (d) Edo, K.; Akiyama, Y.; Saito, K.; Mizugaki, M.; Koide, Y.; Ishida, N. *J. Antibiot*. **1986**, *39*, 1615. Chromophore stereochemistry: (e) Myers, A. G.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc*. **1988**, *110*, 7212.

(5) Myers, A. G.; Hammond, M.; Wu, Y.-S.; Xiang, J.-N.; Harrington, P. M.; Kuo, E. Y. *J. Am. Chem. Soc*. **1996**, *118*, 10006.

(6) Following our disclosure of this transformation,[5] similar conditions for the reductive deoxygenation of 2,3-epoxy alcohols were independently reported by another research group: Dorta, R. L.; Rodríguez, M. S.; Salazar, J. A.; Suárez, E. *Tetrahedron Lett*. **1997**, *38*, 4675.

(7) In studies of the stability of authentic neocarzinostatin chromophore, we observed its rapid decomposition in the presence of 10% HF in acetonitrile ($t_{1/2} \leq 30$ min), but no evidence of its decomposition in the presence of HF·pyridine complex in THF after 2 h at 23 °C (rp-HPLC analysis).

(8) Reviews: (a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl*. **1986**, *25*, 212. (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem*. **1994**, *50*, 21. (c) Schmidt, R. R.; Jung, K.-H. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; Chapter 12.

(9) (a) Schmidt, R. R.; Grundler, G. *Angew. Chem., Int. Ed. Engl*. **1982**, *21*, 781. (b) Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem*. **1984**, 1826. (c) Kinzy, W.; Schmidt, R. R. *Carbohydr. Res*. **1989**, *193*, 33. (d) Boons, G. J. P. H.; Overhand, M.; van der Marel, G. A.; van Boom, J.-H. *Angew. Chem., Int. Ed. Engl*. **1989**, *28*, 1504. (e) Kaneko, T.; Takahashi, K.; Hirama, M. *Heterocycles* **1998**, *47*, 91.

(10) Conditions for the removal of acetonide, cyclopentylidene ketal, and 1,1,3,3-tetraisopropyldisiloxane protective groups for the 1,2-diol, for example, were found to be incompatible with **1**.

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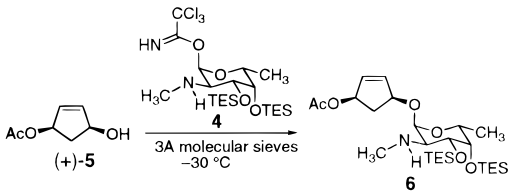
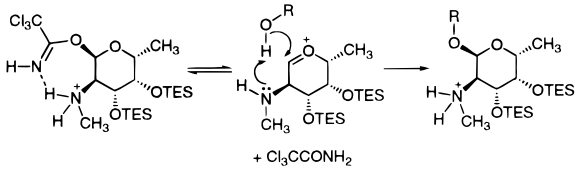
Table 1. Glycosylation of the Model Compound (+)-**5**

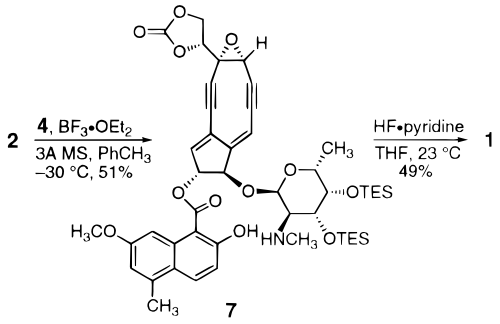


Entry	Catalyst, solvent	Ratio α : β	Yield (%)
1	TfOH, CH_2Cl_2	Exclusively α	82
2	TfOH, $ClCH_2CH_2Cl$	"	70
3	TMSOTf, CH_2Cl_2	"	75
4	BF_3•OEt_2, $PhCH_3$	"	78
5	$Sn(OTf)_2$, Et_2O	11 : 1	73

the glycosylation reaction employing the alcohol (+)-**5** as a model substrate showed that the imidate **4** was an exceptionally effective glycosyl donor (Table 1). Glycosylation reactions were both efficient and rapid, typically proceeding to completion within 1 h at −30 °C. Even more encouraging was the fact that the α-glycoside (**6**) was formed virtually exclusively using a wide variety of Brønsted and Lewis acids (0.2−0.6 equiv) to promote the coupling reaction. The high α-stereoselectivity and reactivity of the glycosyl donor **4** are noteworthy, but no less so than the simple finding that the Schmidt glycosylation can be conducted in the presence of a free amino group. The generality of this finding remains to be explored; a favored mechanistic proposal in the present case invokes an intramolecular ammonium ion−imidate hydrogen bond (seven-membered ring) as the precursor to an anomeric oxonium ion with a (neutral) 2-methylamino group. The glycosyl acceptor may then be directed to the α-face of the oxonium ion by interaction with the amino group (possible hydrogen-bonding/internal basic group).[14]



In light of results from the above model studies, the critical coupling of **4** with the aglycone (**2**) was pursued. A wide variety of conditions was found to bring about the successful coupling of **2** and **4**. Optimal conditions involved reaction at −30 °C using BF_3•OEt_2 as the catalyst and toluene as the solvent, forming exclusively the protected α-glycoside **7** in 51% yield after purification by flash column chromatography. Although the coupled product (**7**) proved to be considerably more stable than the starting aglycone (**2**), this intermediate was typically not stored but was subjected to immediate deprotection. Direct treatment of **7** with HF•pyridine complex[7] in tetrahydrofuran at 23 °C for 1 h in the dark cleaved both TES groups in high yield, as determined by reversed-phase (rp)-HPLC analysis. Purification of the deprotected product by chromatography on Sephadex LH-20 provided pure synthetic neocarzinostatin chromophore ((+)-**1**) in 49% yield, indistinguishable from an authentic sample (TLC, rp-HPLC, UV−vis, IR, CD, ^{1}H NMR). In addition, high-resolution mass spectra of neocarzinostatin chromophore (synthetic) have been obtained for the first time, using an external ion source Fourier transform ion cyclotron resonance (FT-ICR)[15] mass spectrometer equipped with a nanoelectrospray[16] ionization source (calcd for $C_{35}H_{34}NO_{12}$ ([M + H]$^+$), 660.2082; found, 660.2090).[17]



Radiolabeled [*N-methyl*-^{3}H]neocarzinostatin chromophore ((+)-**1**) has also been synthesized. Coupling of the aglycone (**2**) with [*N-methyl*-^{3}H]fucosamine **4** (see Supporting Information) under the optimal conditions previously outlined followed by deprotection provided the radiolabeled chromophore (+)-**1** with a specific activity of 31 mCi/mmol. This product now serves as a radioactive tracer for mechanism of action studies conducted in vivo.

The synthetic route to (+)-**1**, as optimized thus far, involves the convergent assembly of (+)-**2** in 18 steps (average yield per step 87%) from three synthetic precursors that were each prepared with an average yield of 85% per step.[5,18] The final glycosylation and deprotection steps currently proceed in ∼50% yield each. This route provides an alternative source of milligram quantities of (+)-**1** (to the best of our knowledge, no longer available from commercial fermentation sources),[19] as well as the radiolabeled compound.

Acknowledgment. Generous financial support from the National Institutes of Health and Pfizer, Inc., is gratefully acknowledged. We are indebted to Sangwon Lee, Prof. Jesse L. Beauchamp, and the Beckman Institute Resource Center of Mass Spectroscopy (Caltech) for high-resolution mass spectra of synthetic neocarzinostatin chromophore. We also thank Jeffrey G. Varnes for his assistance in the preparation of synthetic intermediates.

Supporting Information Available: Tabulated spectroscopic data for all new synthetic compounds and reproductions of ^{1}H NMR spectra of synthetic and natural neocarzinostatin chromophore (7 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(11) In studies of the glycosylation of a model of neocarzinostatin chromophore, Hirama and co-workers reported the use of a thioglycoside donor containing a 2-*N*-methylammonium group (stoichiometric triflic acid, *N*-iodosuccinimde activation). Interestingly, in this work, only the *β*-glycoside was formed. Takahashi, K.; Tanaka, T.; Suzuki, T.; Hirama, M. *Tetrahedron* **1994**, *50*, 1327.

(12) Glycosidic coupling reactions with free 2-hydroxyl groups are well precedented. For leading references, see: (a) Hanessian, S.; Bacquet, C.; Lehong, N. *Carbohydr. Res.* **1980**, *80*, C17. (b) Hanessian, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; Chapter 16.

(13) Schmidt and co-workers reported that attempted trichloroacetimidate formation from an anomeric hydroxyl group with a free hydroxyl group in the adjacent 2-position produces a tetrahedral adduct and not the imidate (Cinget, F.; Schmidt, R. R. *Synlett* **1993**, 168. Haeckel, R.; Troll, C.; Fischer, H.; Schmidt, R. R. *Synlett* **1994**, 84). IR and ^{1}H and ^{13}C NMR spectra of imidate **4** clearly support the indicated structure, with trichloroacetimidate and 2-*N*-methylamino groups.

(14) Both chair and boatlike transition states are considered viable. Dicationic intermediates (α-ammonium oxonium ions) are considered unlikely, and nucleophilic opening of an aziridinium ion intermediate would produce the *β*-glycoside. When the *N*-TEOC-protected glycosyl donor corresponding to **4** was used in the coupling reaction, the *β* isomer was favored (*β*:α ratio of 2−8:1, varying with reaction conditions).

(15) Rodgers, M. T.; Campbell, S.; Marzluff, E. M.; Beauchamp, J. L. *Int. J. Mass Spectrom. Ion Processes* **1994**, *137*, 121.

(16) Wilm, M.; Mann, M. *Anal. Chem.* **1996**, *68*, 1.

(17) As a testimony to the exceedingly mild nature of this soft ionization technique, the molecular ion peak was also the base peak, with essentially no fragment ion peaks observed (<2% intensity).

(18) (a) Myers, A. G.; Subramanian, V.; Hammond, M. *Tetrahedron Lett.* **1996**, *37*, 587. (b) Myers, A. G.; Hammond, M.; Wu, Y.-S. *Tetrahedron Lett.* **1996**, *37*, 3083.

(19) We thank Kayaku Co., Ltd. for their continued and generous provision of natural neocarzinostatin for our use.