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Development of an Enantioselective Synthetic Route to Neocarzinostatin Chromophore and Its Use for Multiple Radioisotopic Incorporation

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Abstract: A convergent, enantioselective synthetic route to the natural product neocarzinostatin chromophore (**1**) is described. Synthesis of the chromophore aglycon (**2**) was targeted initially. Chemistry previously developed for the synthesis of a neocarzinostatin core model (**4**) failed in the requisite 1,3-transposition of an allylic silyl ether when applied toward the preparation of **2** with use of the more highly oxygenated substrates **27** and **54**. An alternative synthetic plan was therefore developed, based upon a proposed reduction of the epoxy alcohol **58** to form the aglycon **2**, a transformation that was achieved in a novel manner, using a combination of the reagents triphenylphosphine, iodine, and imidazole. The successful route to **1** and **2** began with the convergent coupling of the epoxydiyne **15**, obtained in 9 steps (43% overall yield) from D-glyceraldehyde acetonide, and the cyclopentenone (+)-**14**, prepared in one step (75−85% yield) from the prostaglandin intermediate (+)-**16**, affording the alcohol **22** in 80% yield and with ≥20:1 diastereoselectivity. The alcohol **22** was then converted into the epoxy alcohol **58** in 17 steps with an average yield of 92% and an overall yield of 22%. Key features of this sequence include the diastereoselective Sharpless asymmetric epoxidation of allylic alcohol **81** (98% yield); intramolecular acetylide addition within the epoxy aldehyde **82**, using Masamune's lithium diphenyltetramethyldisilazide base (85% yield); selective esterification of the diol **84** with the naphthoic acid **13** followed by selective cleavage of the chloroacetate protective group in situ to furnish the naphthoic acid ester **85** in 80% yield; and elimination of the tertiary hydroxyl group within intermediate **88** using the Martin sulfurane reagent (79% yield). Reductive transposition of the product epoxy alcohol (**58**) then formed neocarzinostatin chromophore aglycon (**2**, 71% yield). Studies directed toward the glycosylation of **2** focused initially on the preparation of the *N*-methylamino → hydroxyl replacement analogue **3**, an α-D-fucose derivative of neocarzinostatin chromophore, formed in 42% yield by a two-step Schmidt glycosylation−deprotection sequence. For the synthesis of **1**, an extensive search for a suitable 2′-*N*-methylfucosamine glycosyl donor led to the discovery that the reaction of **2** with the trichloroacetimidate **108**, containing a free *N*-methylamino group, formed the α-glycoside **114** selectively in the presence of boron trifluoride diethyl etherate. Subsequent deprotection of **114** under mildly acidic conditions then furnished the labile chromophore (**1**). The synthetic route was readily modified for the preparation of singly and doubly ^{3}H- and ^{14}C-labeled **1**, compounds unavailable by other means, for studies of the mechanism of action of neocarzinostatin in vivo.

Introduction and Background

Neocarzinostatin (NCS) was the first "enediyne" antitumor antibiotic identified and is the prototypical member of the chromoprotein class.[1] Isolated from the culture filtrate of *Streptomyces carzinostaticus*, neocarzinostatin was found to possess broad-spectrum antibiotic activity and showed potent antiproliferative effects in a wide variety of tumor cell lines both in vitro and in vivo.[2] A polymer-conjugated version of the drug (conjugated via its binding protein, vide infra) has been approved for the treatment of cancers of the liver and brain, as well as leukemia, in Japan.[3]

After the discovery of neocarzinostatin, several other members of the newly defined enediyne antibiotic family were identified.[4] Among these are some of the most potent antiproliferative agents known. The family can be broadly divided into a chromoprotein subclass that includes neocarzinostatin, kedarcidin,[5] and C-1027,[6]

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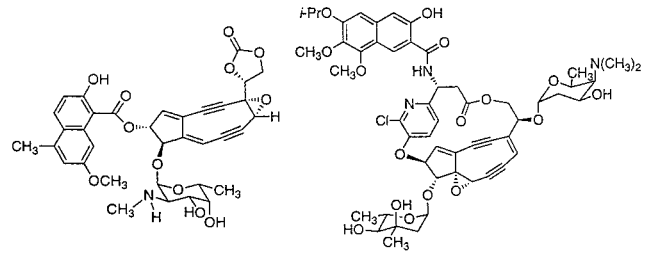
and a nonproteinaceous group, exemplified by the molecules dynemicin A,[7] the calicheamicins,[8] and the esperamicins.[9] Members of the chromoprotein class are composed of a 1:1 complex of a "small molecule" chromophore component and a binding protein, ~11 kD (113 amino acids) in the case of NCS apoprotein. The distinction is critical from the synthetic chemist's perspective, for the chromophoric components of the chromoprotein agents identified thus far are exceedingly unstable in isolation and therefore represent much more challenging targets.[10]

Representative enediyne antibiotics

Chromophoric:



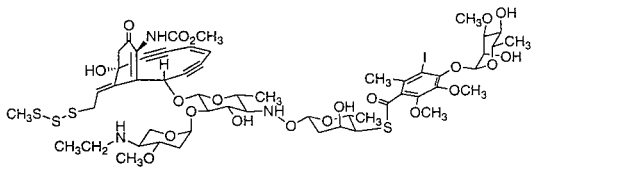
neocarzinostatin chromophore (**1**)

small molecule component of neocarzinostatin protein-chromophore complex

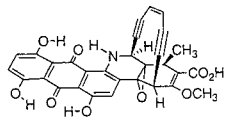
kedarcidin chromophore

small molecule component of kedarcidin protein-chromophore complex

Nonproteinaceous agents:



calicheamicin γ_1



dynemicin A

The structures of the NCS protein−chromophore complex and the NCS apoprotein have been determined by X-ray crystallography. To date, this is the only chromoprotein antibiotic (and, for that matter, the only unmodified enediyne agent) for which such detailed structural information is available.[11] Each π face of the nine-membered epoxy diyne ring contacts the edge of the arene ring of a phenylalanine residue (Phe52 and Phe78) in the binding pocket.[11] The chromophore component (**1**) is readily extracted into organic solvents and is stable under mildly acidic conditions at −70 °C.[12]

The biological activity of NCS is believed to derive from the ability of the chromophore component (**1**) to cleave DNA, a process shown by Goldberg and co-workers to be accelerated ≥1000-fold in the presence of thiols.[13] This acceleration is proposed to arise by a nucleophilic activation process involving addition of thiols to C-12 of the chromophore with concomitant epoxide opening to form a cumulene intermediate that subsequently undergoes biradical-forming cycloaromatization.[14] This proposal is now supported by a large body of evidence, including the direct observation of an unstable cumulene intermediate (methylthioglycolate adduct) and its low-temperature (−38 °C) transformation by cycloaromatization.[15] In the presence of double-stranded DNA, the biradical is proposed to abstract hydrogen atoms from deoxyribose residues of double-stranded DNA, producing mainly single- and a small number of double-stranded DNA cleavage products. There is little sequence selectivity in this process, but it exhibits pronounced base selectivity, with breaks occurring mainly at thymine and adenine residues (T > A ≫ G > C).[16]

The structure of NCS chromophore is highly unusual, characterized most notably by the unsaturated epoxy bicyclo[7.3.0]dodecadienediyne core.[17] The central nine-membered ring of the core is presumably strained, more so than the 10-membered diacetylene rings of, e.g., calicheamicin and dynemicin. Deformation within the nine-membered ring is evident from the crystal structure of NCS;[11] the average C−C≡C bond angle (161.5 ± 1.2°) is significantly distorted from linearity. This distortion presumably contributes to the lability of the chromophore in solution. Once separated from its carrier protein (apo NCS), the chromophore is highly unstable, particularly in the basic pH region, exhibiting a half-life of ~30 s at pH 8.0 (0 °C).[1c] The chromophore is more stable in weakly acidic media and in organic solvents. The half-life of the chromophore in a 1:1 mixture of acetic acid and water is ~5 h at 23 °C. More strongly acidic conditions lead to decomposition of the chromophore.[18] In addition, the chromophore is known to be light-

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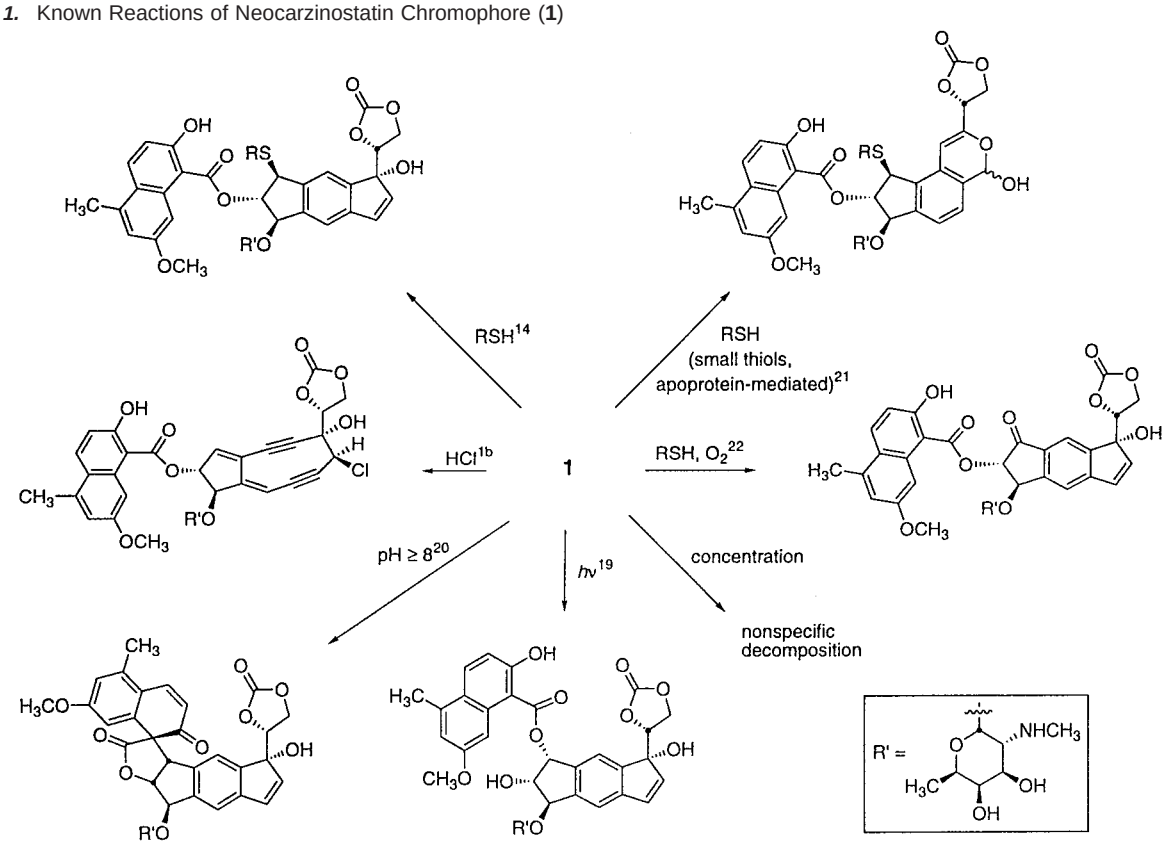
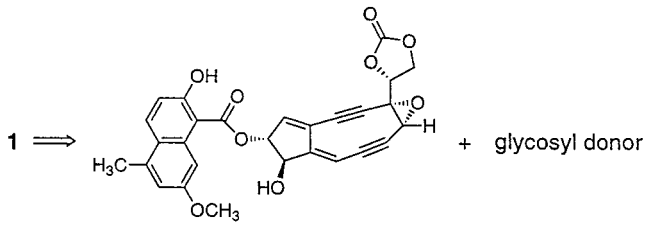
Scheme 1. Known Reactions of Neocarzinostatin Chromophore (**1**)



sensitive,[19] and is especially sensitive toward decomposition in neat form, presumably by free-radical-mediated oligomerization. Most of the known reaction pathways involving **1** are summarized in Scheme 1.[1b,14,20−22]

The significant biological activity, complex structure, and high reactivity of NCS chromophore define it as a compelling, highly challenging synthetic target. The reactivity of the chromophore core in particular complicates the development of a synthetic route to **1**. Although notable achievements in this area have been reported,[23] model studies providing structures containing the nine-membered ring have been few, and these have typically lacked other critical structural elements of **1**.

Some time ago we began a program to synthesize NCS chromophore and study its mechanism of action. Our initial goal was to develop methodology to synthesize the core functionality and then to apply this chemistry to prepare the chromophore aglycon (**2**). The aglycon (**2**) was unknown at the outset of our studies, and remained so until it was synthesized in our laboratory several years later. As a consequence, its stability profile was unknown, a point of some concern, particularly because the aglycon (**2**) was anticipated to be even less stable than the chromophore (**1**), a prediction that was later borne out. Efforts to form the aglycon by hydrolysis of the 2-aminosugar of the natural product failed, not surprisingly, using acidic conditions or any of several commercial glycosidases. Nevertheless, efforts directed toward the laboratory synthesis of this target of undefined but certainly modest stability were pursued in light of the convergence inherent in a route to the synthesis of **1** that would involve the aglycon **2**, as well as the opportunity to study the aglycon mechanistically and to use it for the synthesis of analogues of **1** with modified carbohydrate residues.



neocarzinostatin aglycon (**2**)

Here, we describe in detail the chemistry leading to an enantioselective synthesis of neocarzinostatin chromophore aglycon (**2**) and then of the chromophore itself,[24] to date, the only successful synthesis of any of the chromoprotein chromophore structures. We have adapted our synthetic route to prepare two different radiolabeled forms of **1** and the α-D-fucosyl NCS chromophore analogue **3**, compounds of importance in mechanistic studies of NCS.[25]

(18) For example, the half-life of the chromophore is ~25 min in 50% aqueous acetic acid containing trifluoroacetic acid. Myers, A. G.; Harrington, P. M. Unpublished results. See also ref 54.
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(20) (a) Povirk, L. F.; Goldberg, I. H. *Biochemistry* **1980**, *19*, 4773. (b) Strassinopoulos, A.; Goldberg, I. H. *Biochemistry* **1995**, *34*, 15359.
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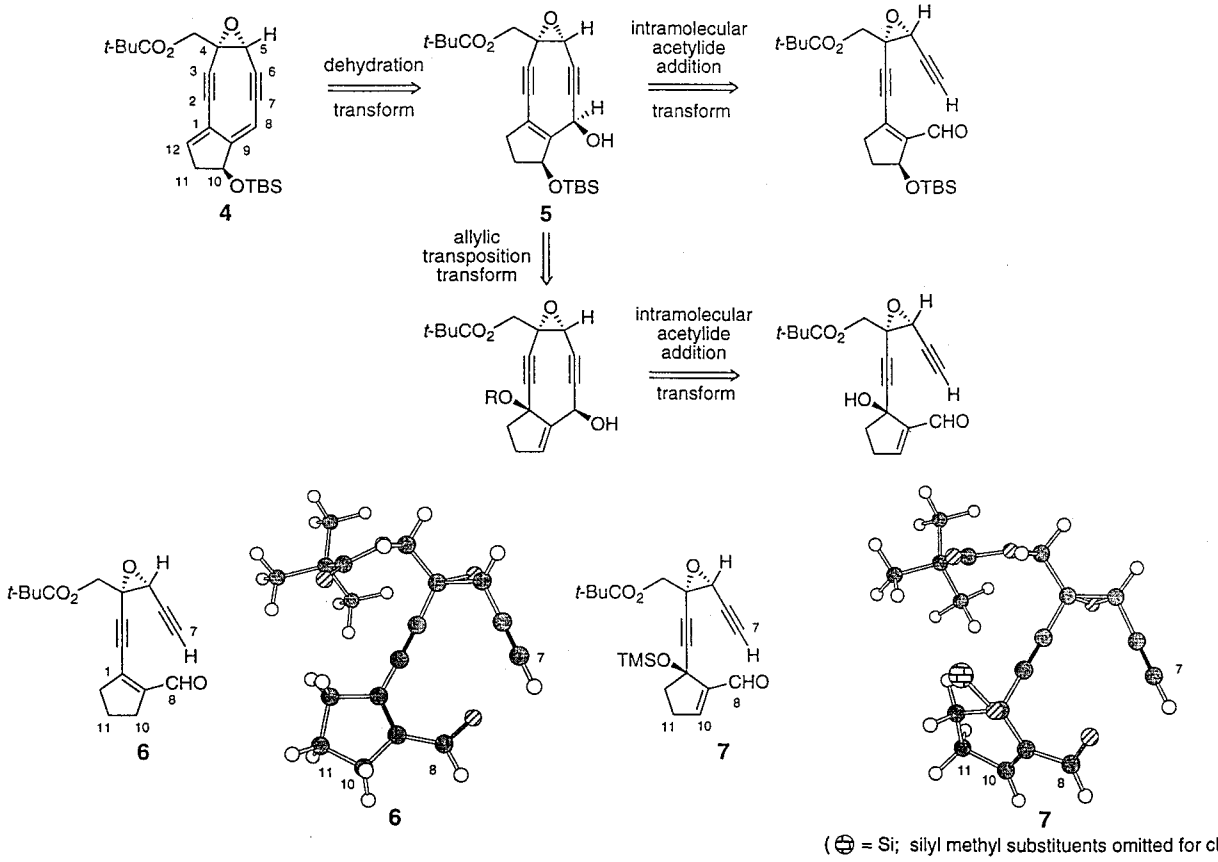
Scheme 2. Retrosynthetic Analysis of a Core Model Featuring an Intramolecular Acetylide Addition Strategy



Early Model Studies

In the initial planning of a synthetic route to the aglycon **2**, it was anticipated that the formation of the reactive and strained nine-membered carbocyclic ring would be a formidable problem. We therefore undertook as a model study the synthesis of a simplified epoxy bicyclo[7.3.0]dienediyne, structure **4** (Scheme 2).[26,27] The C-7−C-8 σ-bond of **4** was identified as a strategic bond in our initial retrosynthetic analysis (more recently, we have been successful in a strategy that targets the transannular C-1−C-9 σ-bond).[23m,23p] When our initial efforts to form the C-7−C-8 bond by the intramolecular insertion of a C-8 vinylidene carbene intermediate into the C−H bond of a C-7 terminal alkyne failed (insertion into the solvent, THF, occurred preferentially), our focus turned toward the formation of this bond by a more conventional method, involving the intramolecular addition of a C-7 localized acetylide anion to the carbonyl carbon (C-8) of an aldehyde group.

To minimize the number of synthetic transformations post ring-closure, it was desirable to conduct the proposed intramolecular acetylide addition in the presence of the epoxide ring. Although we initially had concerns about the feasibility of an epoxy acetylide intermediate (3-chloroacetylides form vinylidene carbenes at low temperature),[28] this aspect of the chemistry did not prove to be problematic. The proposed acetylide−aldehyde addition reaction was not straightforward, however. Now, with the benefit of experience in attempts with many such closures in diverse substrates, it is evident that this is a highly idiosyncratic reaction, whose success depends on favorable kinetics for closure relative to intermolecular acetylide−aldehyde addition (dimerization), as well as other competing reactions, such as the addition of the acetylide-forming base to the aldehyde. Not surprisingly, these additions can be highly

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(27) The conditions (lithium hexamethyldisilazide in the presence of cerium(III) chloride) have been found to be successful in other intramolecular acetylide−aldehyde cyclizations, see: (a) Iida, K.; Hirama, M. *J. Am. Chem. Soc*. **1994**, *116*, 10310. (b) Sato, I.; Akahori, Y.; Iida, K.; Hirama, M. *Tetrahedron Lett*. **1996**, *37*, 5135. (c) Kawata, S.; Yoshimura, F.; Irie, J.; Ehara, H.; Hirama, M. *Synlett* **1997**, 250. (d) Kobayashi, S.; Reddy, R. S.; Sugiura, Y.; Sasaki, D.; Miyagawa, N.; Hirama, M.; Iida, K.; Hirama, M. *J. Am. Chem. Soc*. **2001**, *123*, 2778.

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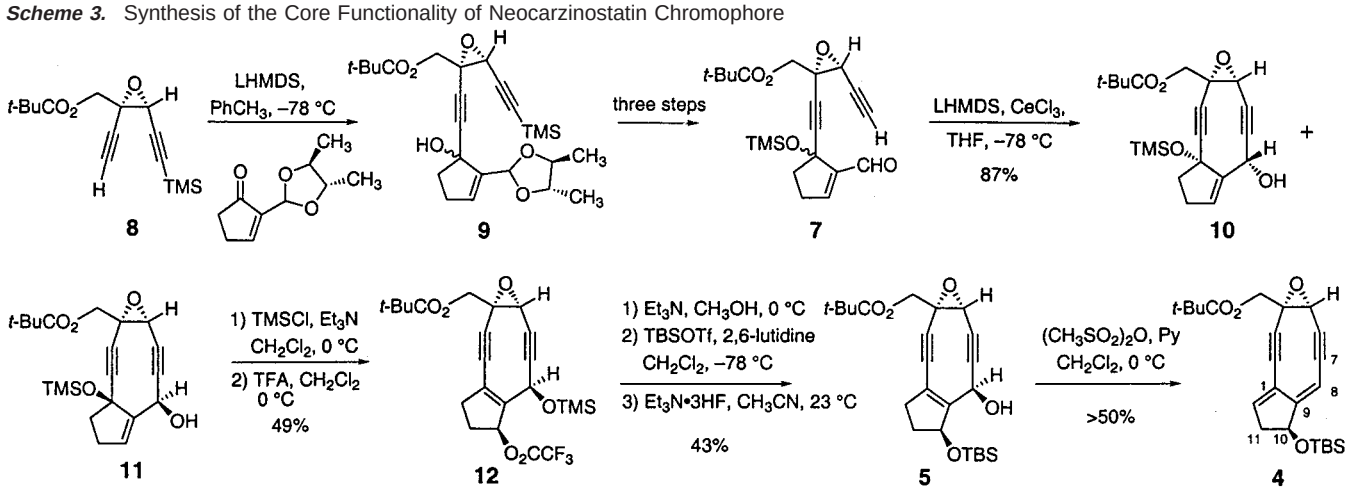
Scheme 3. Synthesis of the Core Functionality of Neocarzinostatin Chromophore

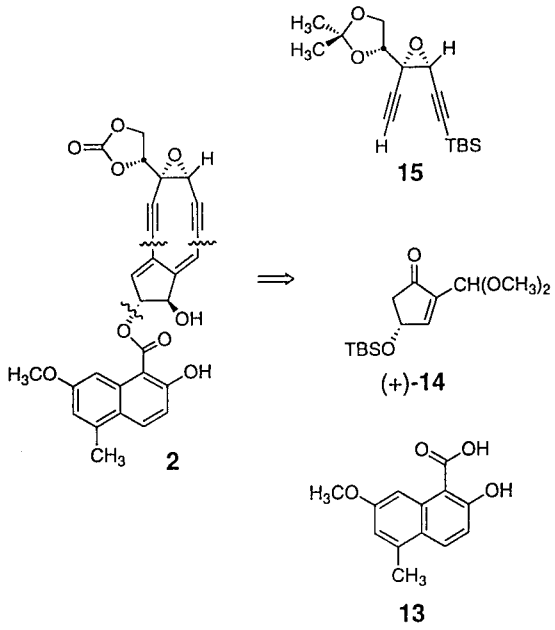


sensitive to the reaction conditions. As exemplified in the discussion that follows, the use of Lewis-acid additives and hindered amide bases can prove critical for successful cyclization to occur.

In the case at hand, the most direct way to apply an intramolecular acetylide−aldehyde addition strategy to synthesize **4** was to hydrate the diene group retrosynthetically by 1,4-addition (structure **5**) and then disconnect the C-7−C-8 σ-bond. Unfortunately, all attempts to bring about the closure reaction within such substrates (e.g., **6**) and, later, more highly oxygenated analogues failed, although macrocyclic dimeric products could be isolated. One possible explanation for the failure of substrate **6** to undergo ring closure involved trajectory analysis, where it was recognized that the planar form of the α,β-unsaturated aldehyde was poorly disposed for intramolecular addition of the acetylide anion (Scheme 2). We reasoned that if C-1 were sp^3-hybridized, rather than sp^2-hybridized, the trajectory for intramolecular acetylide addition would be much more favorable. The proposed hybridization change could be brought about retrosynthetically by 1,4-hydration of the diene of **4** as initially proposed (structure **5**), followed by 1,3-transposition of the resulting allylic silyl ether within **5**. Disconnection of the C-7−C-8 σ-bond after transposition affords a cyclization substrate such as **7**. When implemented, this alternative strategy for closure of the nine-membered ring proved to be successful, but brought with it a 1,3-transposition problem that proved to be the most challenging problem that we were to face in the synthetic route. The alternative cyclization substrate **7** was prepared as shown (Scheme 3), and was found to undergo efficient ring-closure at −78 °C upon treatment with lithium hexamethyldisilazide in the presence of cerium(III) chloride,[26,27] providing the propargylic alcohols **10** and **11** in 87% yield. We were now confronted with the challenge of effecting 1,3-allylic transposition and then 1,4-elimination to form the diene of the target **4**. After much experimentation, a transposition scheme was developed involving exposure of the bis(trimethylsilyl) ether derived from diastereomer **11**[29] to trifluoroacetic acid (0.2 M in dichloromethane, 5 equiv) at 0 °C, producing the trifluoroacetate **12** in 49% yield. Subsequent functional group manipulation provided the allylic alcohol **5**, which underwent 1,4-elimination to afford the epoxydienediyne **4**. As expected, the model dienediyne **4** was highly unstable and, in fact, proved to be less stable than the natural product (**1**). Although we were successful in developing a 1,3-transposition−elimination sequence for the synthesis of the model compound **4**, later, more advanced substrates with C-11-oxygenation did not undergo the solvolytic transposition reaction, or were diverted by internal trapping to produce compounds that could not be used as intermediates for the synthesis of **1** (vide infra). The evolution of the successful pathway to **1** from these initial studies is described in the discussion that follows.

Initial Approaches to the Synthesis of Neocarzinostatin Chromophore Aglycon

Mapping a strategy for the synthesis of the aglycon **2** onto a template suggested by our route to **4**, we envisioned the convergent assembly of **2** from three components: the naphthoic acid **13**, the cyclopentenone **14**, and the epoxydiyne **15**.

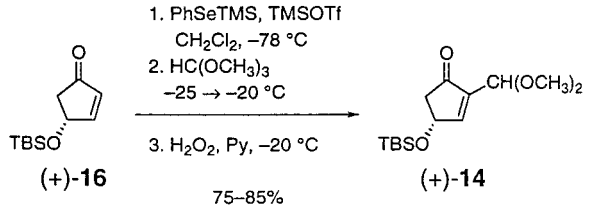


The naphthoic acid component **13** was prepared in six steps in 40−48% yield from 4-bromo-3-methylanisole.[30] The cyclo-

(29) Use of the diastereomer **11** leads to the desired C-10 *S* stereoisomer **12**, by a suprafacial transposition process.

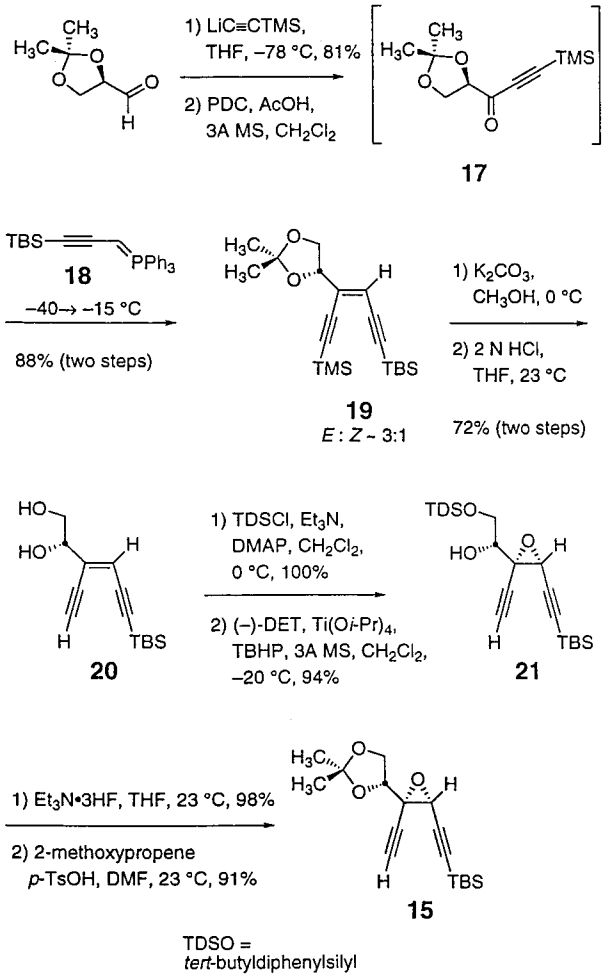
(30) Myers, A. G.; Subramanian, V.; Hammond, M. *Tetrahedron Lett*. **1996**, *37*, 587.

pentenone (+)-**14** was prepared from the well-established prostaglandin intermediate (+)-**16** (>90% ee)[31] in one step by a carefully optimized application of the method of Noyori et al.,[32] involving the sequential addition of trimethylsilylphenyl selenide, trimethyl orthoformate, and hydrogen peroxide−pyridine to (+)-**16**. The timing of the addition of hydrogen peroxide−pyridine to the intermediate β-phenylselenenyl dimethyl acetal, in particular, was critical. If the addition of the oxidant was delayed, decomposition occurred, and if the addition was conducted too rapidly (before the trimethylsilyl enol ether intermediate had reacted completely with added trimethyl orthoformate), then incomplete conversion of the starting cyclopentenone ((+)-**16)** was observed. The enantiomeric purity of (+)-**14** was determined to be ≥90% ee by a sequence involving 1,2-reduction with DIBAL, isolation of the major (cis) diastereomer, Mosher esterification, and ^{1}H NMR analysis.



The synthesis of the epoxydiyne component **15** is shown in Scheme 4. Addition of lithium (trimethylsilyl)acetylide to D-glyceraldehyde acetonide[33] produced a diastereomeric mixture of propargylic alcohols (ratio 1.3:1, 81% yield). Oxidation of the secondary alcohol gave the corresponding propargylic ketone **17**, which was added directly (in solution) to the ylide **18**, affording a mixture of the olefins **19** (~3:1), in which the desired trans isomer (with the acetylenic substituents cis) predominated, in 88% yield. The isomers were not separated at this stage, but instead the trimethylsilyl and acetonide protective groups were removed by sequential treatment with potassium carbonate in methanol followed by hydrochloric acid (2 N, aqueous tetrahydrofuran). The desired diol (**20**) was then isolated in isomerically pure form by flash column chromatography (72% yield). Attempts to use the diol **20** as a substrate in the Sharpless asymmetric epoxidation reaction (SAE)[34] provided further evidence that the stereochemical outcome in SAE of allylic diols is difficult to predict.[35] No reaction was observed when **20** was treated under catalytic or stoichiometric SAE conditions[36] employing (−)-diethyl D-tartrate, while the epoxidation with (+)-diethyl L-tartrate as ligand (stoichiometric conditions) afforded the β-epoxide in quantitative yield. This epoxide was not useful for the synthesis of **1**. This problem was easily circumvented by the selective silylation of the primary alcohol of **20** with *tert*-butyldiphenylsilyl chloride (TDSCl, quantitative yield); using the resulting silyl ether as substrate, SAE with (−)-diethyl tartrate as ligand (stoichiometric conditions) provided the required α-epoxide (**21**) in 94% yield. Cleavage of

Scheme 4

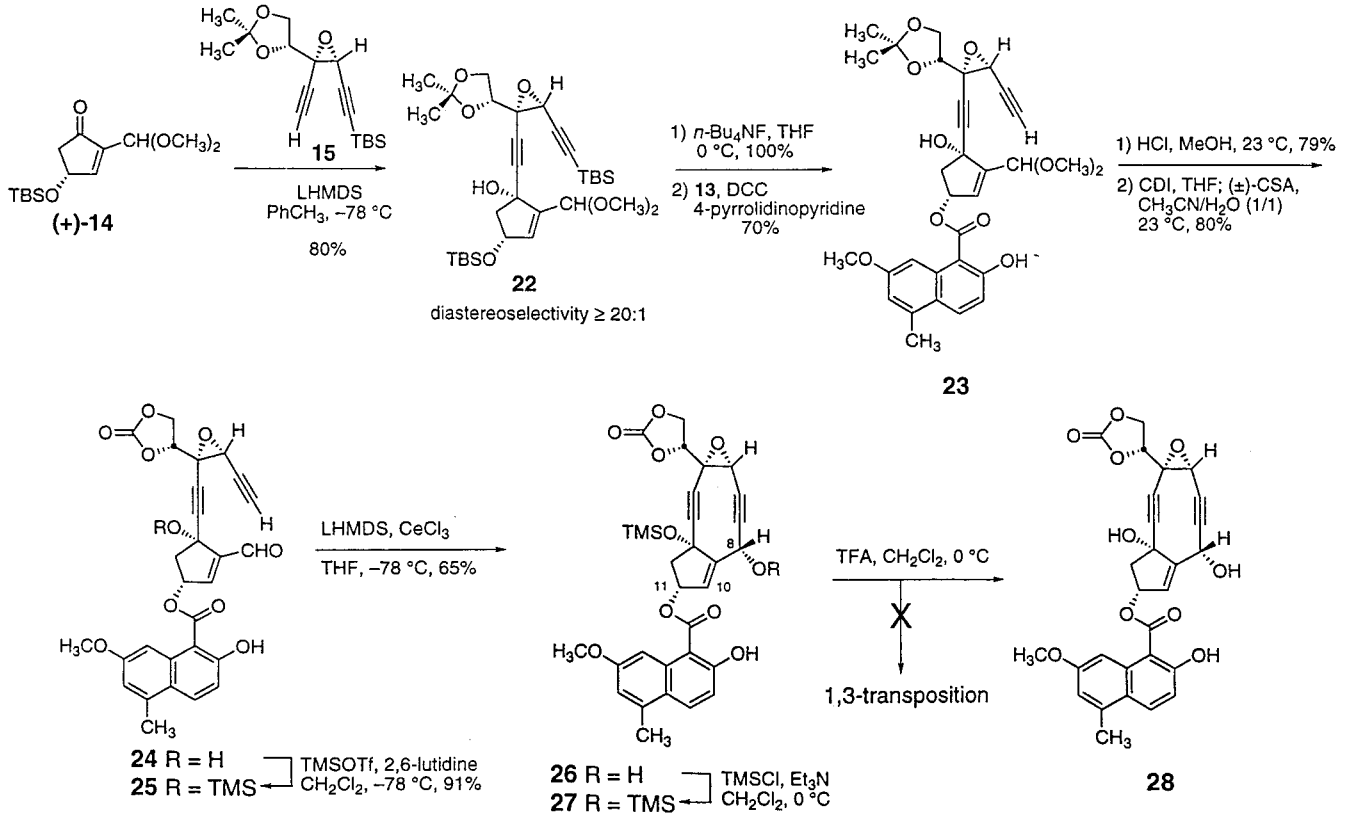


the silyl ether occurred upon exposure of **21** to triethylamine trihydrofluoride, affording the corresponding diol epoxide as a pale yellow solid in 98% yield (≥95% ee). Treatment of this diol with 2-methoxypropene in the presence of catalytic amounts of *p*-toluenesulfonic acid then afforded the acetonide **15** as a white solid.

With components **13**−**15** in hand, studies directed toward the synthesis of neocarzinostatin chromophore aglycon (**2**) along the lines established with the core model structure **4** were initiated (Scheme 5). Deprotonation of the epoxydiyne **15** with lithium hexamethyldisilazide (LHMDS) followed by addition of the enone **14** to the resulting anion afforded the adduct **22** in 80% yield and with ≥20:1 selectivity for the diastereomer shown (attack opposite the *tert*-butyldimethylsilyl ether substituent). Treatment of the adduct (**22**) with tetrabutylammonium fluoride (TBAF) cleanly removed both silyl groups, providing the corresponding diol in quantitative yield. Selective esterification of the secondary alcohol with the naphthoic acid **13** in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine or 4-(dimethylamino)pyridine (DMAP) as catalyst provided the monoester **23** in 70% yield. The acetonide protective group was then cleaved in the presence of concentrated hydrochloric acid in methanol, producing the corresponding tetraol in 79% yield, and the ethylene carbonate group was introduced by using carbonyldiimidazole (CDI) in tetrahydrofuran. Exposure of the crude reaction mixture to (±)-CSA cleaved acylimidazolide intermediates that had formed, as well

(31) Myers, A. G.; Hammond, M.; Wu, Y. *Tetrahedron Lett.* **1996**, *37*, 3083.
(32) Suzuki, M.; Kawagishi, T.; Noyori, R. *Tetrahedron Lett.* **1981**, *22*, 1809.
(33) Schmid, C. R.; Bryant, J. D.; Dowlatzedah, M.; Philips, J. L.; Prather, D. E.; Schantz, R. D.; Sear, N. L.; Vianco, C. S. *J. Org. Chem.* **1991**, *56*, 4056.
(34) Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974.
(35) (a) Takano, S.; Iwabuchi, Y.; Ogasawara, K. *Synlett* **1991**, *2*, 548. (b) Takano, S.; Setoh, M.; Takahashi, M.; Ogasawara, K. *Tetrahedron Lett.* **1992**, *33*, 5365.
(36) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

Scheme 5



as the dimethyl acetal group, affording the α,β-unsaturated aldehyde **24** in 80% yield. Preparatory to ring closure, the tertiary hydroxyl group of **24** was protected by using trimethylsilyl triflate and 2,6-lutidine to provide the corresponding trimethylsilyl ether **25** in 91% yield.

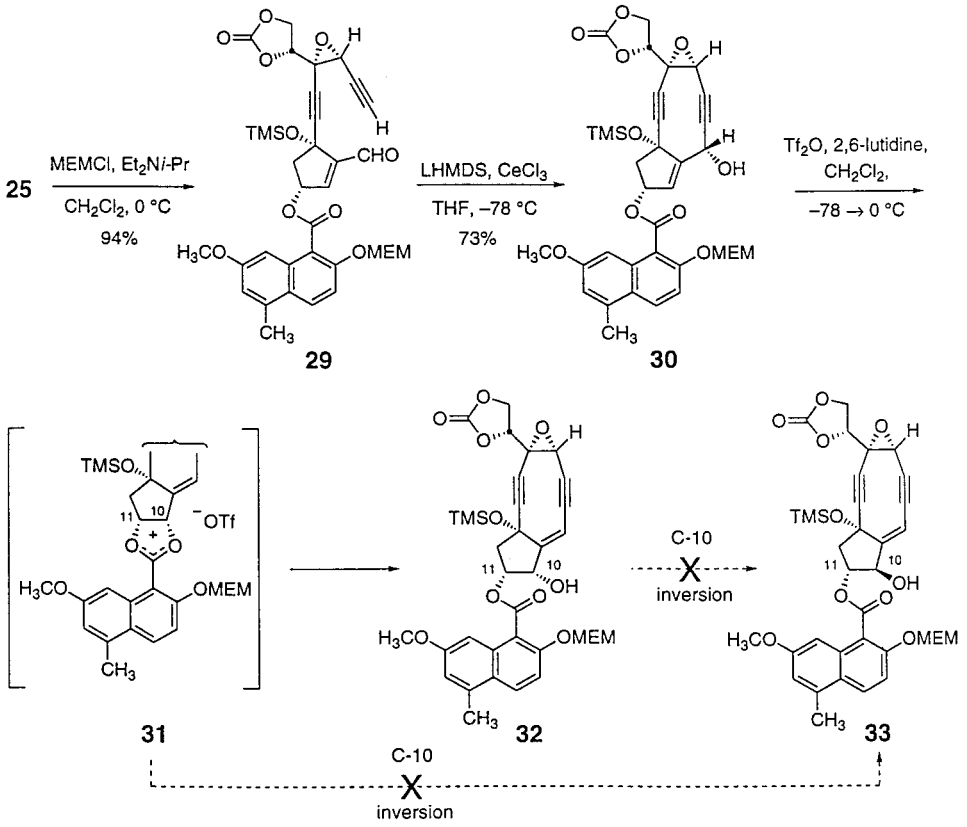
Conditions developed for the closure of the nine-membered ring in the synthesis of the model compound **4** were successfully applied to the more elaborate substrate **25**. Thus, treatment of a suspension of anhydrous cerium(III) chloride (3 equiv) and the intermediate **25** in tetrahydrofuran at −78 °C with LHMDS (1 equiv) provided the ring-closure product **26** in 50−65% yield.[26,27] The lower yield of cyclized product (**26**) relative to the earlier model study was attributed to competitive base-induced cleavage of the ethylene carbonate in both the product and the starting material. As expected, the cyclic product (**26**) was a somewhat sensitive material, particularly in neat form, although not to the degree exhibited by **1**. The alcohol **26** could be concentrated and held in neat form for brief periods of time without significant decomposition, and could be stored for several days in a frozen benzene matrix at −20 °C.

Treatment of the alcohol **26** with excess trimethylsilyl chloride and triethylamine in dichloromethane furnished the bis-silyl ether **27**, which was isolated after aqueous workup and then exposed to trifluoroacetic acid in dichloromethane at 0 °C in an effort to effect allylic transposition, as before. However, the only products observed under these conditions were those resulting from cleavage of the silyl ether bonds. Consequently, alternative means to bring about the necessary 1,3-allylic transposition were explored.

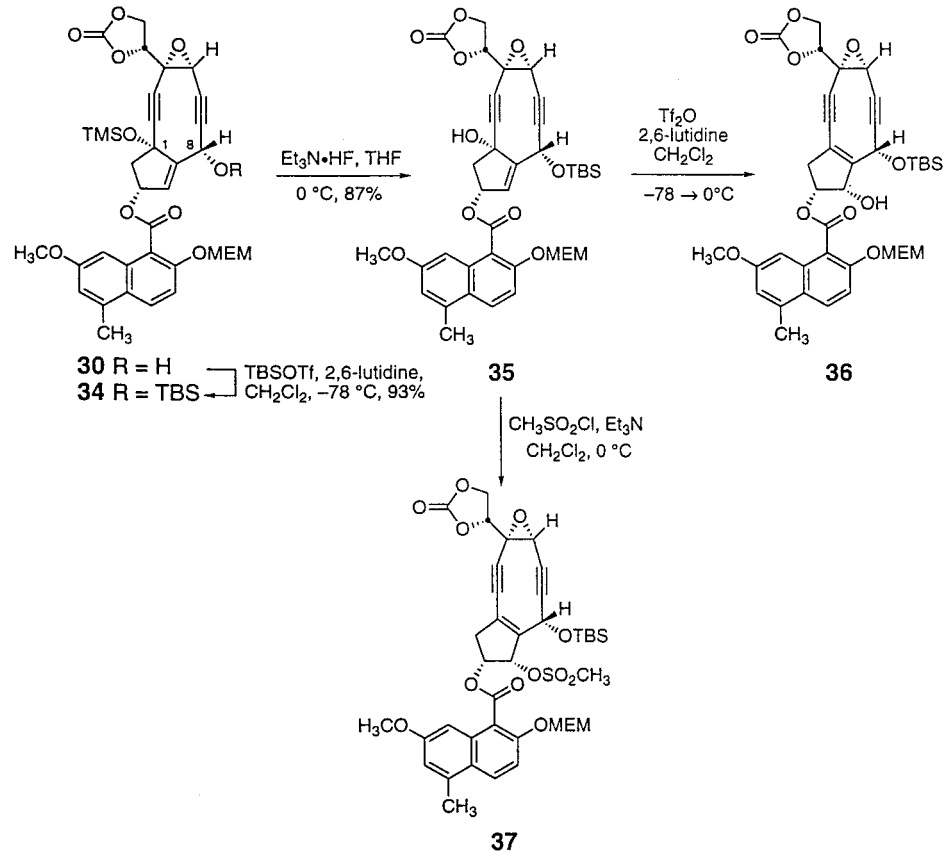
One alternative that was investigated involved activation of the C-8 hydroxyl group toward solvolysis by triflation. To accomplish this the naphthol group was protected first, prior to cyclization, as the corresponding methoxyethoxymethyl (MEM) ether **29** (Scheme 6, 94% yield). Addition of LHMDS to a suspension of cerium(III) chloride and **29** in THF at −78 °C provided the corresponding cyclic product **30** in 73% yield.[26,27] Treatment of **30** with triflic anhydride and pyridine afforded a product that was exceedingly unstable, decomposing within minutes in neat form and within hours in solution (benzene). ^{1}H NMR analysis of the chromatographically purified product indicated that allylic transposition had indeed occurred; however, the C-10 hydroxyl group of the product (**32**) was determined to have *S* stereochemistry (cis to the C-11 naphthoic acid ester), and not the *R* stereochemistry (trans to the C-11 naphthoic acid ester, see **33**) found in **1** and **2**. The stereochemical outcome of this transposition was rationalized as arising from neighboring-group participation of the C-11 naphthoic acid ester. Addition of water to the proposed dioxolenium ion intermediate **31** (Scheme 6) and selective expulsion of the C-10 hydroxyl group (or expulsion of the C-11 hydroxyl group followed by acyl transfer) would then afford the transposed product **32**. Minor signals attributed to the C-10 ester were observed in the ^{1}H NMR spectrum of the product **32**.

Although the necessary 1,3-transposition reaction had occurred, a stereochemical inversion (at C-10) was now necessary to make use of this product for the synthesis of **1**. Several experiments were conducted in an effort to trap the intermediate **31** at C-10 with an external, oxygen-centered nucleophile such as acetate or trifluoroacetate, without success. Efforts to invert the C-10 stereocenter of the rearranged product **32** were also unsuccessful. The instability of **32** greatly complicated both strategies and, ultimately, led us to conclude that this series of compounds was simply too unstable to complete the synthesis of **2** and **1**. We then shifted our focus to efforts to transpose the

Scheme 6

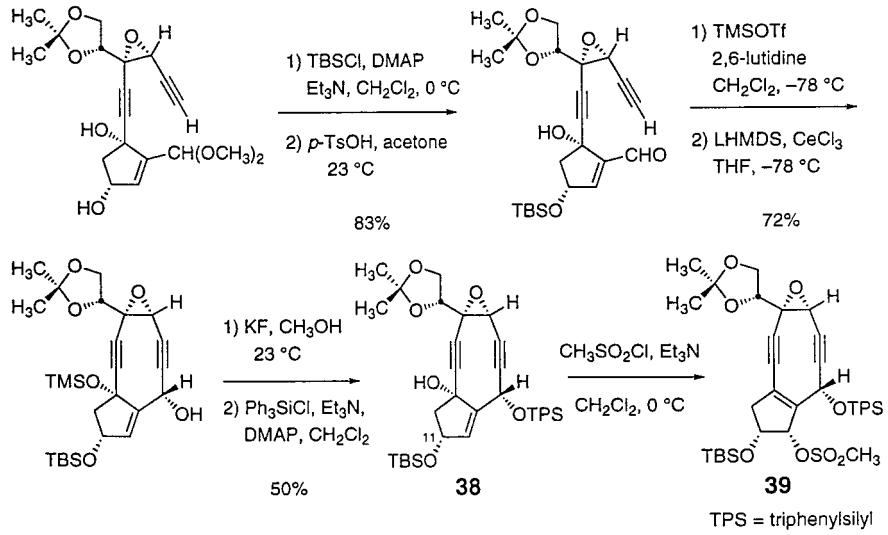


Scheme 7

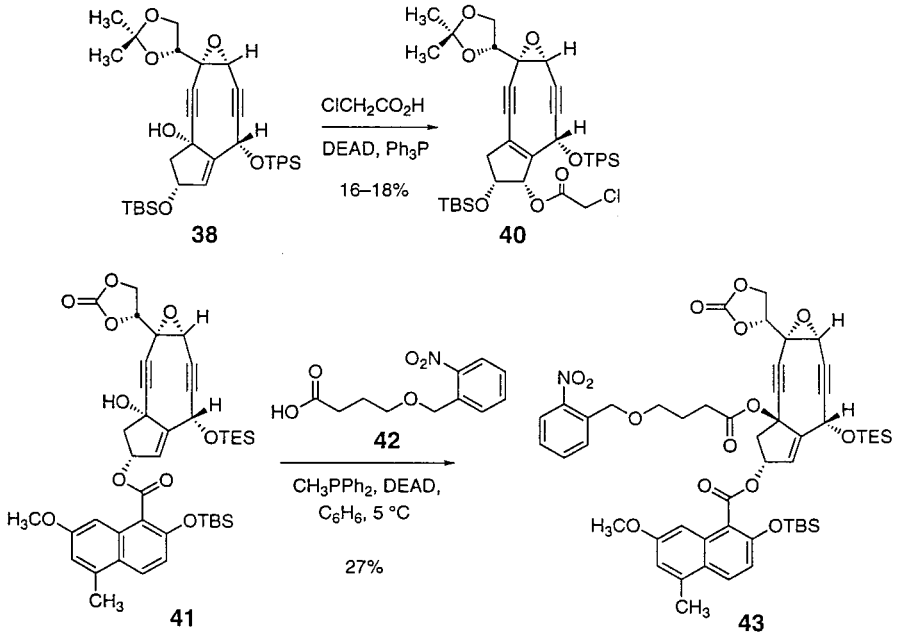


C-1 hydroxyl group rather than the C-8 hydroxyl group, with the expectation that the products in this series would be more easily handled as a consequence of the presumed greater stability of the tetrasubstituted olefin (Scheme 7).

Scheme 8



Scheme 9

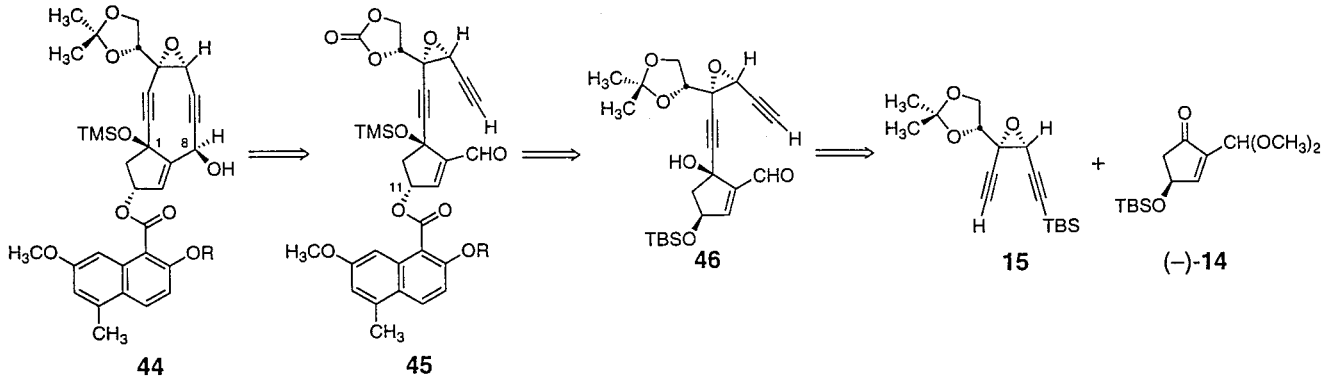


To activate the C-1 hydroxyl group for transposition, the C-8 hydroxyl group was first protected as the corresponding *tert*-butyldimethylsilyl ether (**34**, 93% yield, Scheme 7); selective cleavage of the trimethylsilyl ether was then effected by exposure of **34** to triethylamine trihydrofluoride, affording the tertiary alcohol **35** in 87% yield. Treatment of **35** with triflic anhydride in the presence of 2,6-lutidine afforded the transposed product **36**, with cis stereochemistry again, presumably as a consequence of neighboring-group participation. Unfortunately, the transposed alcohol **36** was not significantly more stable than the alcohol **32**, undergoing rapid decomposition both in neat form and in solution. As before, efforts to invert the C-10 hydroxyl group, now within intermediate **36**, or to trap the presumed intermediate dioxolenium ion by external nucleophilic additions, were not successful.

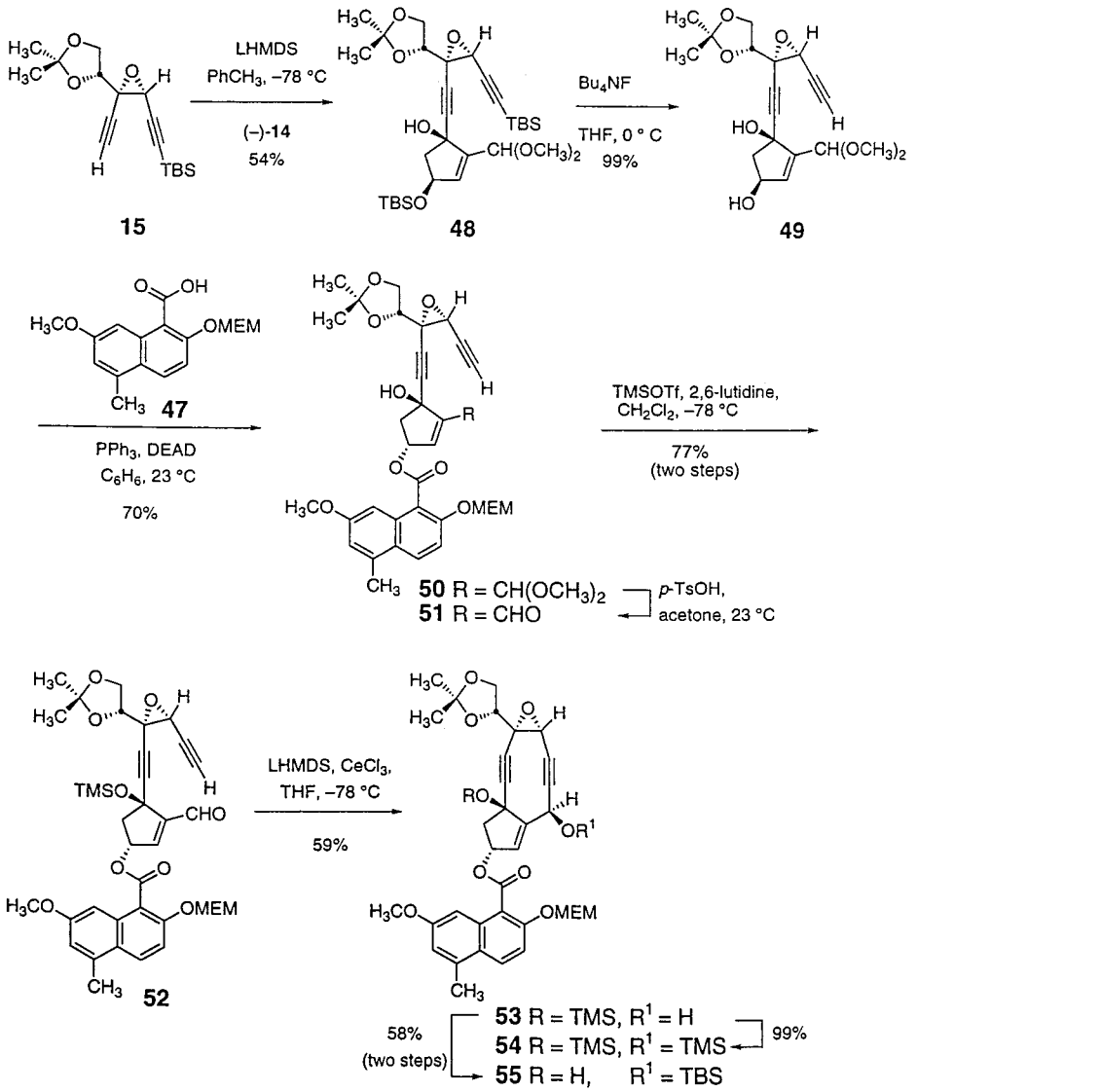
Interestingly, treatment of alcohol **35** with methanesulfonyl chloride and triethylamine led to direct transposition to form the allylic mesylate **37**, also with cis stereochemistry. The mesylate was slightly more stable than the rearranged alcohol **36**, and seemed to be an ideal candidate for direct nucleophilic inversion; however, efforts to accomplish this under a broad spectrum of conditions led only to decomposition.

To examine the role of the naphthoic acid ester in the rearrangement chemistry, we prepared the substrate **38** with a *tert*-butyldimethylsilyl ether at C-11 in place of the naphthoic acid ester (Scheme 8). Not unexpectedly, the behavior of the substrate **38** toward triflic anhydride was markedly different than that of substrate **35**, bearing a C-11 naphthoic acid ester. Treatment of **38** with triflic anhydride and pyridine resulted in rapid and nonspecific decomposition of the starting material. The reactivity of **38** toward methanesulfonyl chloride, on the other hand, paralleled that of substrate **35**, affording the (unstable) rearranged mesylate **39**. Attempted invertive displacement of the mesylate **39** was unsuccessful with use of several different nucleophiles; only decomposition of the substrate was observed. Allylic transposition of the tertiary allylic alcohols **38** (with a C-11 silyl ether) and **41** (with a C-11 naphthoic acid ester) was also attempted under Mitsunobu conditions (Scheme

Scheme 10

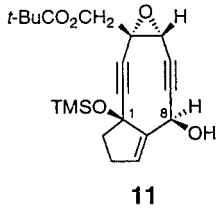


Scheme 11

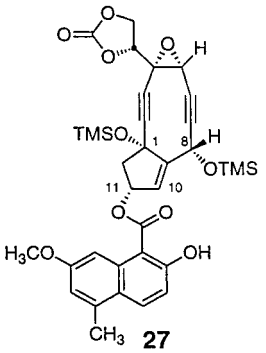


9), although little precedent exists for the application of the Mitsunobu reaction for the inversion of tertiary alcohols or for 1,3-transposition of allylic alcohols.[37] Reaction of alcohol **38** with chloroacetic acid, triphenylphosphine, and diethyl azodicarboxylate (DEAD) afforded the rearranged diester **40** in 16−18% yield, whereas the more functionalized substrate **41** afforded the product of direct invertive displacement of the tertiary hydroxyl group (**43**), under substantially modified Mitsunobu conditions (methyldiphenylphosphine, DEAD, γ-hydroxybutyric acid *o*-nitrobenzyl ether **42**); neither transformation was of apparent use for our purpose.

In considering alternative strategies to achieve the necessary allylic transposition reaction, we returned to our earlier model structure (**11**) that had rearranged successfully (after trimethylsilylation of the secondary alcohol) in the presence of trifluoroacetic acid (**11** → **12**, Scheme 3). As described, attempted extension of this strategy to the more complex substrate **27** had failed, presumably due to C-11 oxygenation



11



27

within this substrate. It occurred to us that an additional distinction between two substrates was the stereochemical variation at C-1 and C-8. This led us to consider the rearrangement of the diastereomeric substrate **44**, whose retrosynthesis is outlined in Scheme 10.

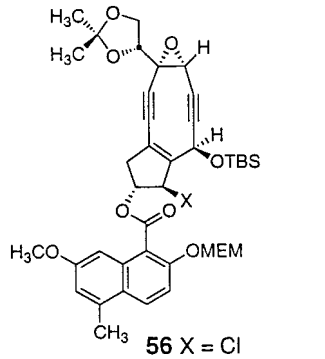
It was expected that intramolecular acetylide addition within the C-1 epimeric epoxy aldehyde substrate **45** would occur in analogy to prior closures in the diastereomeric ring series, via the *s*-trans conformation of the α,β-unsaturated aldehyde (**45** → **44**, Scheme 10). We planned to introduce the C-11 naphthoic acid ester of **45** by Mitsunobu inversion of the secondary alcohol derived from intermediate **46**.[37] Intermediate **46** would be accessed by diastereoselective addition of the lithium acetylide derived from **15** to (−)-**14**, the enantiomer of the substrate used for the preparation of the previous diastereomeric series. The basic elements of this plan were successfully pursued to obtain the diastereomeric cyclization product **53** and its differentially silylated forms **54** and **55**, as shown in Scheme 11. Interestingly, closure of the nine-membered ring required a large excess of LHMDS (9 equiv) to proceed to completion, and the product (**53**) was found to be much less stable than the diastereomeric ring-closure products **27** or **30**, decomposing within a few days even when stored in a solid benzene matrix at −20 °C.

The instability of intermediates **53** → **55** in this series greatly complicated their manipulation and purification, and consequently the yields for intermediates beyond **53** were difficult to determine accurately.

In contrast to the behavior of the diastereomeric alcohol **35**, the alcohol **55** underwent nonspecific decomposition upon treatment with triflic anhydride. Interestingly, the reaction of alcohol **55** with methanesulfonyl chloride or methanesulfonic anhydride afforded products whose ^{1}H NMR spectra were consistent with structures assigned as the chloride **56** and the mesylate **57**, respectively. Although these products apparently possessed the requisite trans stereochemistry between the C-10 and C-11 centers, we were unable to develop a means for the conversion of either of these compounds to intermediates useful for the synthesis of **2**. More practically, the marked instability of this diastereomeric series convinced us to return to the initial diastereomeric series for the continuation of our studies.

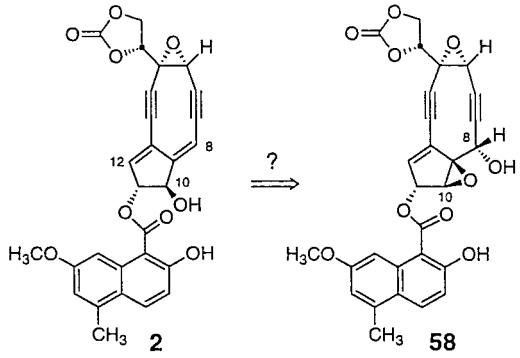
Enantioselective Synthesis of Neocarzinostatin Chromophore Aglycon

Having exhausted many potential routes for the preparation of the substituted epoxy dienediyne core of **1** by the transposition of the allylic alcohols discussed in the previous section, attention was turned to a new strategy involving the bis-epoxide **58** as a potential precursor to neocarzinostatin chromophore aglycon (**2**).



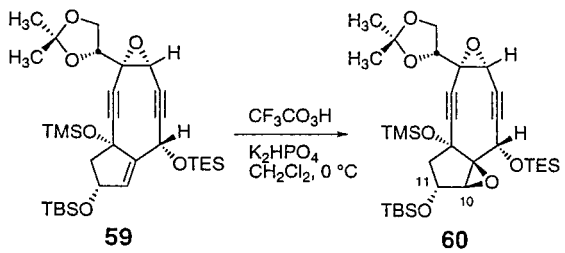
56 X = Cl
57 X = OSO_2CH_3

Many possible means of accomplishing the necessary reduction of **58** to form **2** were envisioned, although that which ultimately proved successful was discovered serendipitously (vide infra).



2 58

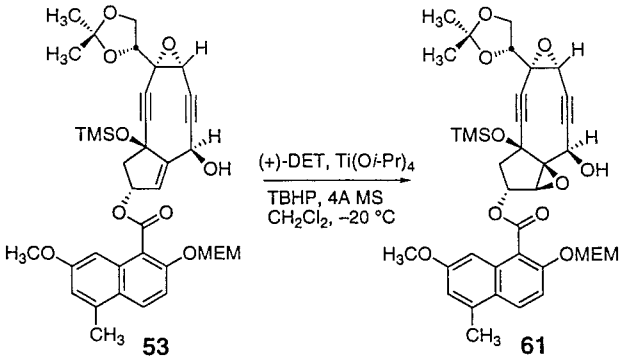
The most direct means by which to access the intermediate **58** involved the epoxidation of substrates in hand, and this was investigated first. A number of reagents were screened for the epoxidation of the substrate **59**, for example, *m*-CPBA, dimethyldioxirane, and methyl(trifluoromethyl)dioxirane,[38] and the peroxycarboximidic acid derived from the combination of 90% hydrogen peroxide and acetonitrile,[39] none of which was successful. Only buffered trifluoroperoxyacetic acid afforded the desired epoxide (**60**). The 0-Hz coupling constant between the protons at C-10 and C-11 was diagnostic for a trans relationship, as expected if epoxidation had occurred from the less hindered face of the olefin within **59**. However, the low yield of the epoxide product (significant decomposition of the substrate and/or product occurred) prompted us to seek an improved method to introduce the epoxide.



59 60

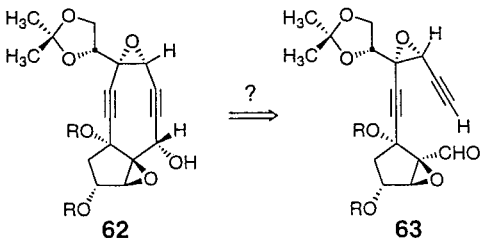
(37) (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) Hughes, R. L. *Org. React.* **1992**, *42*, 335.
(38) (a) Murry, R. W.; Jeyaraman, R. *J. Org. Chem.* **1985**, *50*, 2847. (b) Mello, R.; Fiorentino, M.; Sciacovelli, O.; Curci, R. *J. Org. Chem.* **1988**, *53*, 3980. (c) Adam, W.; Bialis, J.; Hadjiarapolou, L. *Chem. Ber.* **1991**, *124*, 2377.
(39) Gagieu, C. H.; Grouiller, A. V. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1009.

We were able to direct the epoxidation reaction by using the hydroxyl group at C-8 in the diastereomeric series of intermediates (in which the C-1 and C-8 centers were inverted); for example, alcohol **53** was epoxidized under SAE conditions with use of (+)-DET to afford the epoxide **61**.[36] The purification of

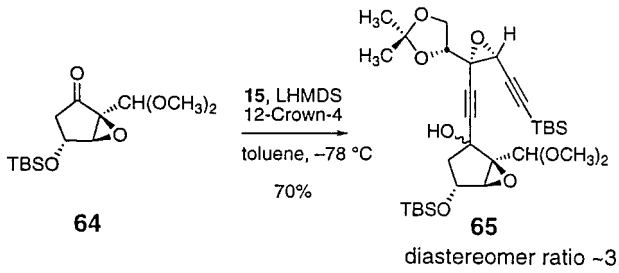


61 from residual (+)-DET was difficult, and the instability of the precursors made material throughput a challenge. Consequently, introduction of the epoxide prior to closure of the nine-membered ring was pursued, although there were concerns about the influence the epoxide would have upon the closure reaction; the outcome was not easily predicted (see **62** ⟹ **63**).

Our first approach to the synthesis of epoxy aldehyde intermediates having the general structure **63** involved the coupling of the epoxy diyne component **15** with the epoxy ketone **64**, prepared from the reaction of (+)-**14** with alkaline hydrogen peroxide (91% yield, ≥95% de, ≥85% ee). Addition of the acetylide anion derived from **15** and LHMDS to the ketone **64** provided a 1:1 mixture of the diastereomers **65** in 10−15% yield. Although modification of the reaction conditions

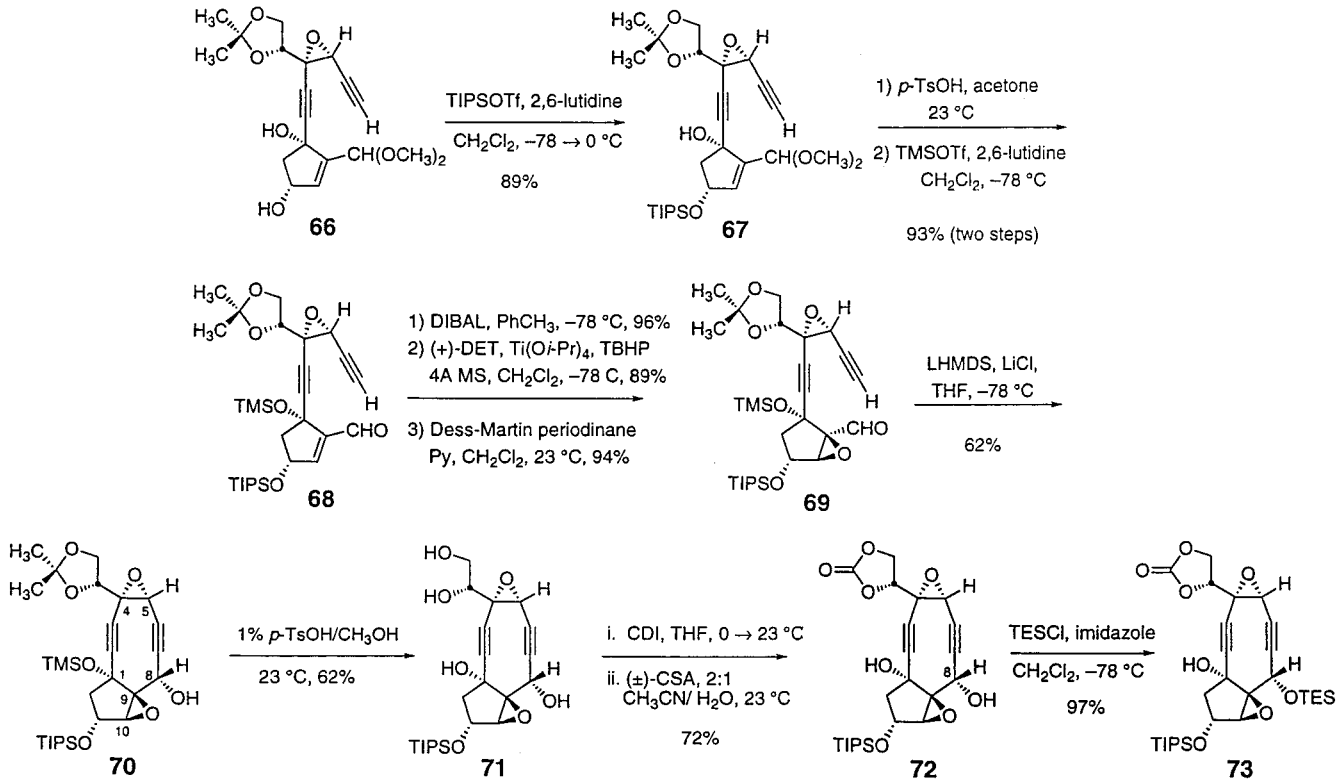


led to an improvement in the yield of the coupling reaction (12-Crown-4 as an additive, 70%), the diastereoselectivity remained unacceptably low (~3:1).



Diastereoselective introduction of the second epoxy group within **63** was best achieved by application of the SAE, as shown in Scheme 12, beginning with the prior intermediate **66**. Selective silylation of **66** with triisopropylsilyl triflate provided the secondary silyl ether **67** in 89% yield. Hydrolysis of the dimethyl acetal group within this product with *p*-toluenesulfonic acid (*p*-TSA) in acetone afforded the corresponding aldehyde. Protection of the tertiary alcohol with trimethylsilyl triflate then furnished the aldehyde **68** in 93% yield from **67**. The aldehyde **68** underwent 1,2-reduction upon treatment with DIBAL,

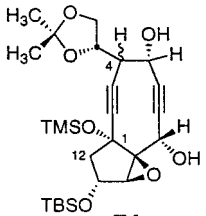
Scheme 12



affording the corresponding allylic alcohol in 96% yield. This product was epoxidized diastereoselectively under SAE conditions with use of (+)-DET, providing the corresponding bis-epoxy alcohol in 89% yield; oxidation with Dess−Martin periodinane[40] buffered with pyridine then afforded the bis-epoxy aldehyde **69** in 94% yield.

Closure of the nine-membered ring could be brought about by treatment of epoxy aldehyde **69** with LHMDS in the presence of cerium(III) chloride, but higher conversions and cleaner reactions were observed with this substrate when anhydrous lithium chloride was used in lieu of cerium(III) chloride. Thus, addition of LHMDS to a suspension of the aldehyde **69** and lithium chloride (50 equiv) in THF at −78 °C furnished the cyclic alcohol **70** in 62% yield. An unexpected, but highly noteworthy feature of the product **70** was its stability to chromatography, concentration, and routine manipulations. This stands in marked contrast to the "olefinic" series of cyclization products which, as mentioned, was found to be exceedingly difficult to handle. The stereochemistry of the addition reaction was confirmed to be as shown (**70**) by the preparation of a cyclic disiloxane derivative linking the C-1 and C-8 hydroxyl groups.

Exploratory studies to effect the reductive fragmentation step, using the epoxy alcohol **70** or a closely related substrate, suggested that the C-4−C-5 epoxide was more labile to reductive cleavage than the C-9−C-10 epoxide. For example, ^{1}H NMR analysis of the product mixture from attempted Barton deoxygenation, using the thiocarbonylimidazolide derived from **70**,[41] provided evidence that the C-4−C-5 epoxide had opened, whereas the C-9−C-10 epoxide remained intact. Similar results were obtained by using the one-electron reductant Cp_2TiCl of RajanBabu and Nugent[42] (the alcohol **74**, a single diastereomer of undetermined stereochemistry at C-4, was isolated).



74

We therefore elected to introduce the C-1−C-12 double bond prior to reduction to favor cleavage of the desired epoxide; the ethylene carbonate group was also introduced concurrently (Schemes 12 and 13). Thus, treatment of the alcohol **70** with methanolic *p*-TSA provided the tetraol **71** in 62% yield, which afforded the carbonate **72** upon reaction with CDI in THF at 0−23 °C (72% yield). The secondary alcohol was protected as the corresponding triethylsilyl ether (97% yield) without competing silylation of the tertiary hydroxyl group.

Among many conditions examined for elimination with the tertiary alcohol **73**, optimal results were obtained by using the Martin sulfurane as the dehydrating agent,[43] producing the olefin **75** in 93% yield (Scheme 13). This product and all similar compounds containing the cyclic ene−yne core were notably more sensitive intermediates, darkening within minutes upon concentration. These compounds could be held in neat form only for brief periods, but were stable to storage at −20 °C in a frozen benzene matrix for several days.

Reexamination of the Cp_2TiCl reagent for the reductive fragmentation, now within the substrate **75**, once again led to selective opening of the C-4−C-5 epoxide, as well as cleavage of the cyclic carbonate, forming the triol **76**. Efforts to transform **77** into a halohydrin intermediate (as a substrate for subsequent reduction) by selective cleavage of the allylic C−O bond were also not successful; again, the major reaction path involved opening of the C-4−C-5 epoxide. A thorough study of ionic hydrogenation conditions[44] led only to the realization that both epoxy groups within **75** were unexpectedly resilient under acidic conditions; exposure of **75** to trifluoroacetic acid−triethylsilane, for example, provided only the alcohol **77**, by acidic cleavage of the triethylsilyl ether. This transformation was ultimately used productively, albeit with more conventional reagents ((±)-CSA in aqueous acetonitrile, 92%).

With a slightly altered focus, we attempted to transform the alcohol **77** into the corresponding secondary iodide, intending to effect reductive epoxide opening by subsequent lithium−halogen exchange. Accordingly, **77** was treated with triphenylphosphine, iodine, and imidazole in dichloromethane at −10 °C (Scheme 14).[45] The product of this reaction proved to be highly unstable. After much experimentation, a suitable procedure for isolation of the product was found, a key element being the direct loading of the reaction mixture onto a pre-slurried column of silica gel immediately upon completion of the reaction (TLC analysis). The isolated product was found not to be the expected epoxy iodide, but instead the epoxydienediyne **78**.[46] Further support for this assignment was obtained by ^{1}H NMR analysis of the corresponding triethylsilyl ether (**79**), a derivative that provided well-resolved, fully assignable proton resonances. Although the secondary iodide expected from the reaction may be an intermediate in the formation of the epoxy dienediyne **78**, it was not observed. The transformation of epoxy iodides into allylic alcohols under nucleophilic conditions is known, but generally requires more forcing conditions than those employed here.

With the somewhat serendipitous discovery of a viable protocol to prepare the epoxy dienediyne functionality of neocarzinostatin, the product **78** representing the first fully oxygenated neocarzinostatin core structure to be synthesized, we sought to incorporate the naphthoic acid ester and thus complete the synthesis of the aglycon (**2**). The observed instability of the intermediate **78** suggested that the introduction of the ester at this stage or beyond would not be feasible. To allow for naphthoic acid ester formation prior to reductive transposition, small adjustments in the hydroxyl group protection scheme were introduced (Scheme 15), the primary modification being the substitution of *tert*-butyldimethylsilyl for triisopropylsilyl (**69** → **82**). This permitted masking of the C-8 hydroxyl

(40) (a) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899. (c) Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, *59*, 7549.

(41) Barton, D. H. R.; Motherwell, R. S. H.; Motherwell, W. B. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2363.

(42) (a) RajanBabu, T. V.; Nugent, W. A.; Beattie, M. S. *J. Am. Chem. Soc.* **1990**, *112*, 6408. (b) Rajanbabu, T. V.; Nugent, W. A. *J. Am. Chem. Soc.* **1994**, *116*, 986.

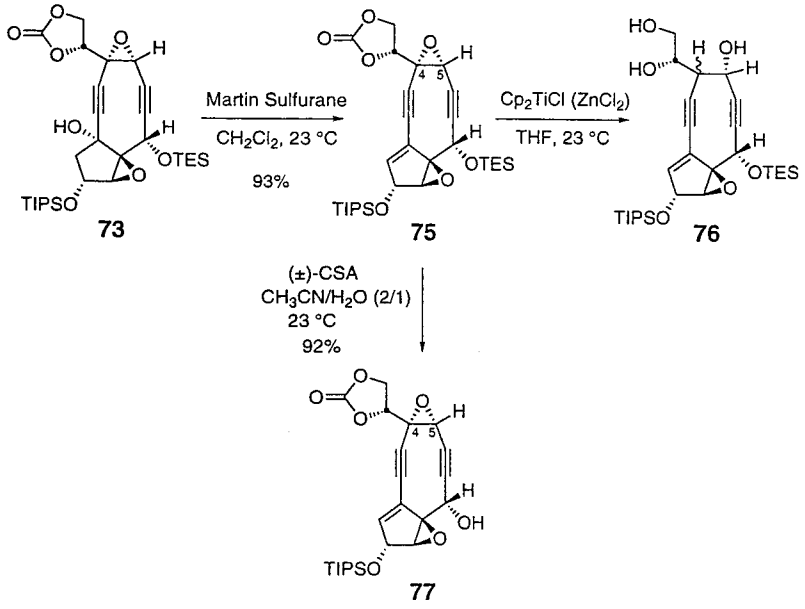
(43) (a) Martin, J. C.; Arhart, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 4327. (b) Arhart, R. J.; Martin, J. C. *J. Am. Chem. Soc.* **1972**, *94*, 4997.

(44) For a review of ionic dehydrogenations in organic synthesis, see: Kursamov, D. V.; Parnes, Z. N.; Loim, N. M. *Synthesis* **1974**, 634.

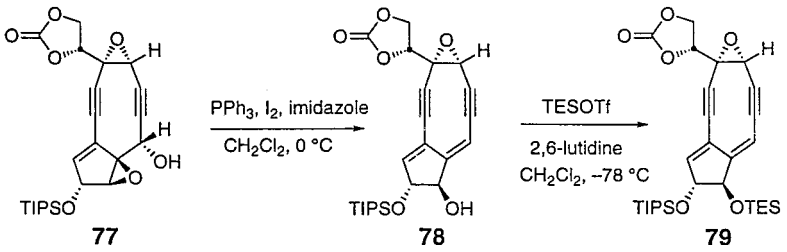
(45) Garegg, P. J.; Samuelsson, B. J. *J. Chem. Soc., Chem. Commun.* **1979**, 978.

(46) More recently, this reductive deoxygenation procedure was reported independently by another research group: Dorta, R. L.; Rodríguez, M. S.; Salazar, J. A.; Suárez, E. *Tetrahedron Lett.* **1997**, *38*, 4675.

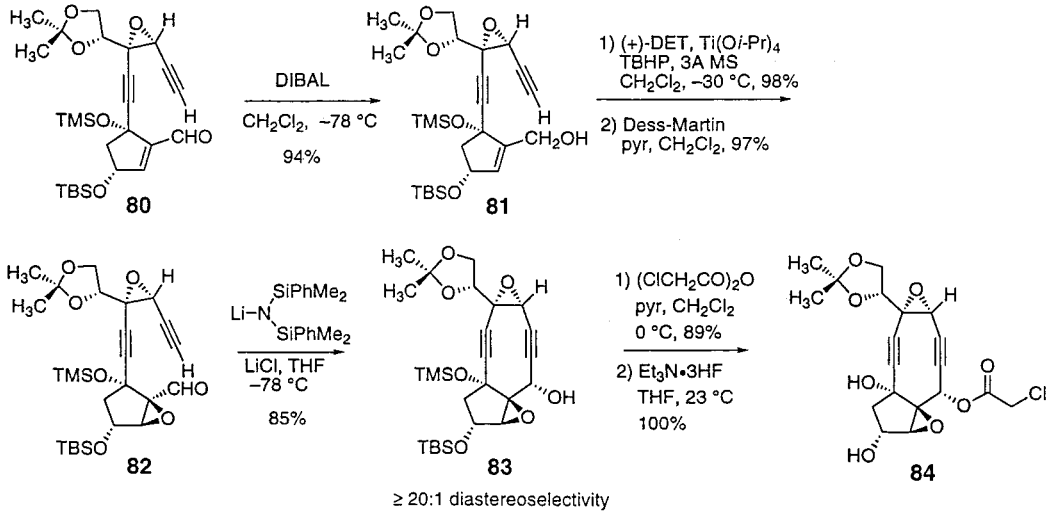
Scheme 13



Scheme 14



Scheme 15

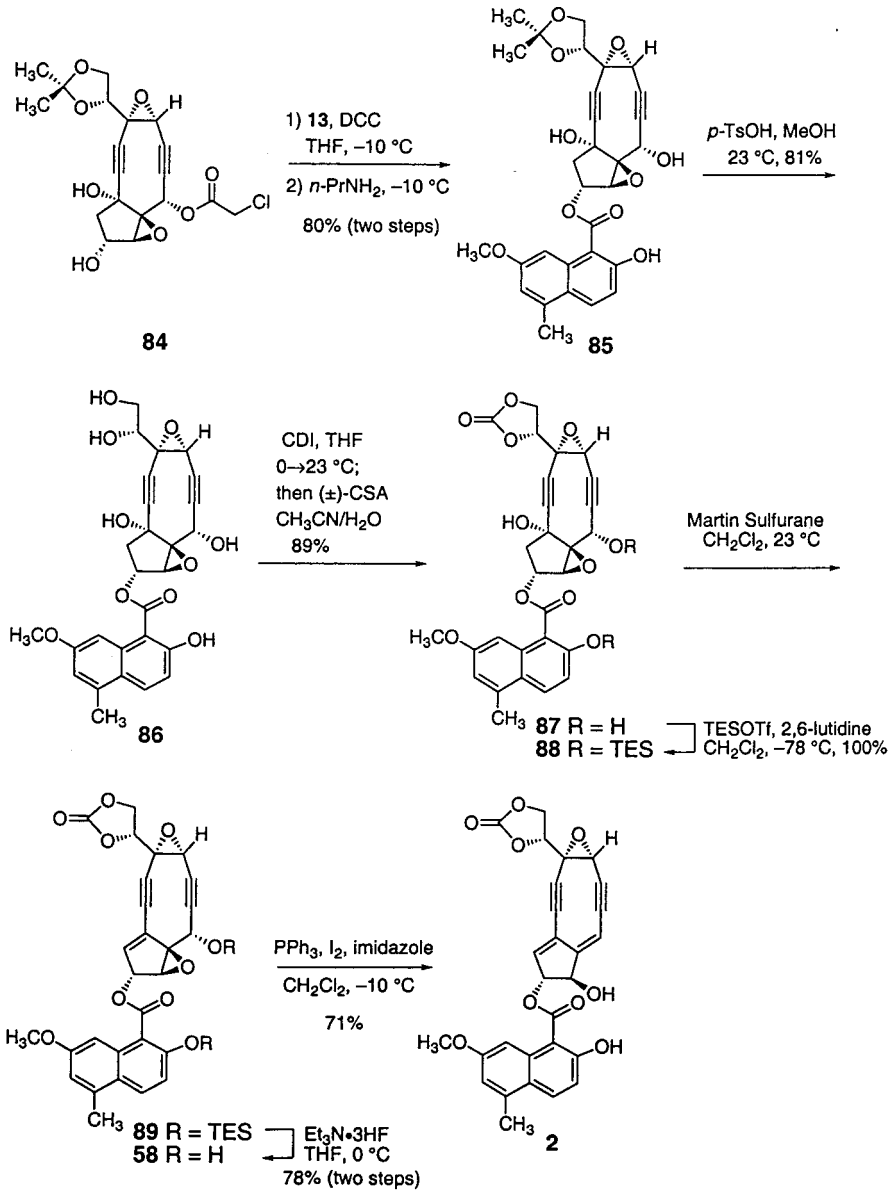


group as a chloroacetate ester, an orthogonal protective group that was stable to conditions necessary for the cleavage of the C-11 silyl ether, yet was removable under mild conditions. The only other significant modification of prior chemistry within Scheme 15 is the use of Masamune's hindered base, lithium bis(dimethylphenylsilyl)azide,[47] for the ring-closure reaction (**82** → **83**), a modification that was necessitated by the poor conversions observed with use of LHMDS as base. This subtle yet critical variation in the cyclization protocol serves to highlight the delicate interplay of relative reaction rates that can determine the success or failure in these reactions (here, presumably the rate of deprotonation by the base versus the rate of its 1,2-addition to the aldehyde).

With an efficient route to the diol **84**, selective acylation of the secondary hydroxyl group with the naphthoic acid **13** was examined. Optimum results were obtained upon combination of **84** and **13** (3 equiv) with DCC (5 equiv) in THF at −10 °C followed by the addition of *n*-propylamine to the crude reaction

(47) (a) Zhinkin, D. Y.; Malnova, G. N.; Gorislavskaya, Zh. V. *Zh. Obshch. Khim.* **1968**, *38*, 2800. (b) Masamune, S.; Ellingboe, J. W.; Choy, W. *J. Am. Chem. Soc.* **1982**, *104*, 5526.

Scheme 16

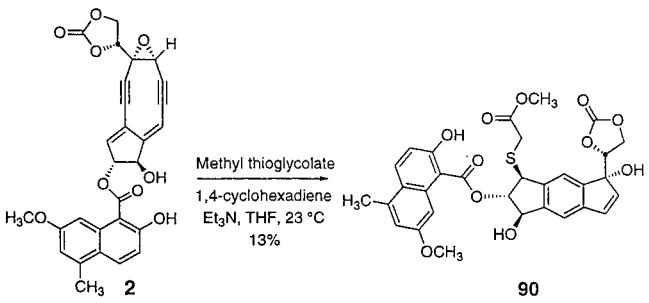


mixture to bring about cleavage of the chloroacetate ester in situ (Scheme 16). Under these conditions, the ester **85** was obtained in 80% yield. Considerable experimentation was required for the development of this procedure, for the naphthoic acid **13** was prone to self-coupling. Competing acylation of the tertiary hydroxyl group of **84** was also observed. The selection of the reaction solvent and the coupling reagent proved to be essential elements for the success of this reaction, with DCC in THF emerging as a uniquely successful combination.

To complete the synthesis of the aglycon **2**, the acetonide protective group of **85** was cleaved in the presence of methanolic *p*-TSA to provide the pentaol **86** in 81% yield; the ethylene carbonate group was then introduced as before, by reaction with CDI in THF, affording the cyclic carbonate **87** in 89% yield (Scheme 16). The naphthol and secondary hydroxyl groups were protected simultaneously with use of triethylsilyl triflate-2,6-lutidine, (quantative yield) and the resulting bis(triethylsilyl) ether (**88**) was treated with the Martin sulfurane reagent to induce elimination of the tertiary hydroxyl group, furnishing the olefinic product **89** in 79% yield. Removal of the triethylsilyl groups within the product **89** in the presence of triethylamine trihydrofluoride then provided the epoxy alcohol **58** in 99% yield.

Treatment of the epoxy alcohol **58** with a mixture of triphenylphosphine, iodine, and imidazole led directly to the highly unstable aglycon **2**. Close monitoring of the reaction by TLC showed that the transformation was complete within 5 min at −10 °C. As before, no intermediates were detectable in the reaction. Workup involved quenching excess triphenylphosphine−iodine with methanol (50 equiv), followed by the addition of pentane to precipitate triphenylphosphine oxide and imidazolium iodide. Flash column chromatography was conducted at 0 °C (cold room) and the pooled aglycon fractions were concentrated at 0 °C by using a rotary evaporator with an ice bath. All manipulations (chromatography, venting after concentration, etc.) were conducted under argon. Also, Kishi's free-radical inhibitor (5-*tert*-butyl-4-hydroxy-2-methylphenyl sulfide)[48] was added to all solutions, to suppress decomposition. Typically, test tubes used for fraction collection during aglycon purification contained a solution of Kishi inhibitor in deoxy-

genated benzene (50 mL, 2.5 mg/mL). With these procedures for synthesis and purification, the optically pure aglycon (**2**) was obtained in up to 71% yield. Typically, 2−3 mg batches of neocarzinostatin chromophore aglycon (**2**) were prepared by this protocol.

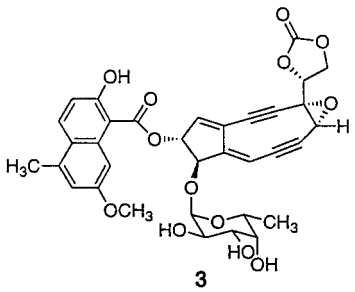


The aglycon (**2**) was observed to degrade much more rapidly than neocarzinostatin chromophore (**1**), both in solution and in neat form. Synthetic **2** provided good quality ^{1}H NMR and CD spectra, but its half-life in solution was too short to obtain a suitable ^{13}C NMR spectrum. All efforts to obtain a mass spectrum of **2** in isolation with a variety of soft-ionization techniques also failed, almost certainly due to the instability of **2**. We were successful in obtaining mass spectral data by precomplexation of the synthetic aglycon (**2**) with purified neocarzinostatin apoprotein in water (pH 7); peaks consistent with the protein-complexed aglycon were observed. For further confirmation of the structure, the aglycon (**2**) was treated with methyl thioglycolate (75 equiv), triethylamine (75 equiv), and 1,4-cyclohexadiene (150 equiv) in THF at 23 °C, affording the thiol adduct **90** in analogy to experiments with neocarzinostatin chromophore (**1**). Triethylamine was required in the latter experiment, providing support for the proposed participation of the amino group of the carbohydrate residue as an internal base in the thiol activation of **1**.[25,49]

Glycosylation Studies

To complete the synthesis of neocarzinostatin chromophore (**1**), the problem of stereoselective glycosylation of the aglycon (**2**) was addressed. Direct glycosylation of **2** in unprotected form had been planned as part of our initial retrosynthetic analysis. The instability of the aglycon (**2**) limited the options available for glycosylation conditions, however, and the instability of the final product (**1**) restricted the selection of protective groups for the amino and hydroxyl groups of the carbohydrate coupling partner. It was known that the chromophore (**1**) was particularly tolerant of mildly acidic conditions; for example, solutions of **1** in neat acetic acid showed no evidence of decomposition within 1 d at 23 °C.[50] Mildly acidic solutions of fluoride ion were deemed to be particularly worthy of consideration for final deprotection. These were defined more precisely in control experiments with **1**. Exposure of **1** to 10% (v/v) aqueous hydrofluoric acid in acetonitrile at 23 °C led to rapid decomposition ($t_{1/2} \leq 30$ min), but the milder reagent hydrogen fluoride−pyridine complex in THF was well tolerated (no evidence of decomposition of **1** after 2 h at 23 °C, rp-HPLC analysis). Groups that were labile under the latter conditions were therefore targeted for protection of the carbohydrate residue.

To begin our glycosylation studies, we elected to pursue the synthesis of the α-D-fucose analogue **3** prior to addressing the more difficult problem of **1** itself. Compounds **3** and **1** differ only in the nature of the 2′-substituent of the carbohydrate residue, a distinction that was of interest from mechanistic as well as synthetic perspectives. The 2′-methylamino substituent of **1** was presumed to make it a more challenging target than **3** (with a 2′-hydroxyl group), a prediction that was later confirmed.

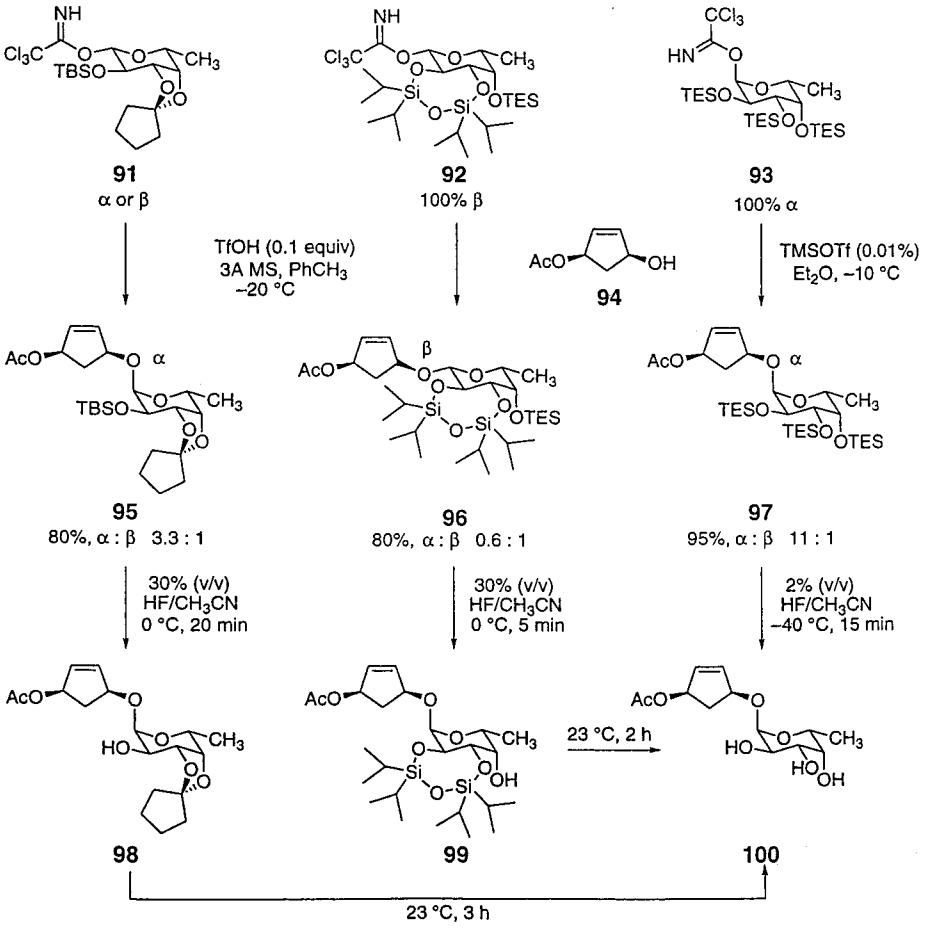


The critical issues to be resolved in any glycosylation reaction are the method of carbohydrate activation, selectivity in the glycosidic bond formation (α versus β), and the nature of the protective groups as they pertain to the latter issue and, in the case of **1** and **3**, to conditions for deprotection vis-à-vis product stability. The Schmidt trichloroacetimidate glycosyl activation method was recognized at the outset to be nearly ideal in the context of our synthetic problem, as it has been in so many complex carbohydrate syntheses, largely for the mildly acidic conditions that are used to promote the coupling reaction, as well as the opportunities to alter α/β selectivity by modification of the trichloroacetimidate stereochemistry and/or the coupling conditions.[51]

Three differently protected trichloroacetimidate-activated D-fucose donors were prepared (**91**, **92**, and **93**, see Supporting Information) and examined as substrates for the synthesis of protected fucosyl glycosides to select an optimum substrate for the synthesis of **3**. The cyclopentenol **94** was used in lieu of the aglycon **2** in initial studies; results of glycosylation reactions with this substrate, as well as subsequent deprotection experiments, are depicted in Scheme 17. Briefly, we learned that silyl ethers, and triethylsilyl ethers in particular, were well suited for protection of the hydroxyl groups of the carbohydrate coupling partner. Ketals (acetonide, cyclopentylidene) were too robust to liberate **1** or **3** intact, as was the disiloxane group within substrate **92**. Also, the β-selectivity observed in coupling with the latter substrate suggested that bulky substituents in the 2′-position were to be avoided if α-selectivity was desired, a proposal that has proven to be somewhat general throughout this work. Among the three substrates, the tris(triethylsilyl) ether **93** was clearly superior in terms of α-selectivity in product formation (further improved upon use of 3 equiv of **93** and trimethylsilyl triflate as catalyst) and the mildness of the

(48) Kishi, Y.; Aratani, M.; Tanino, H.; Fukuyama, T.; Goto, T.; Inoue, S.; Sugiura, S.; Kakoi, H. *J. Chem. Soc., Chem. Commun.* **1972**, 64.
(49) (a) Myers, A. G.; Kort, M. E.; Hammond, M. *J. Am Chem. Soc.* **1997**, *119*, 2965. See also ref 24.
(50) Myers, A. G.; Harrington, P. M. Unpublished results. See also ref 54.
(51) Reviews: (a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212. (b) Schmidt, R. R. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: Oxford, 1991; Vol. 6, p 33. (c) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21. (d) Schmidt, R. R.; Jung, K.-H. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; Chapter 12.

Scheme 17



subsequent deprotection conditions and so was selected for coupling with **2**.

Operationally, synthesis of the α-fucosyl analogue of neocarzinostatin chromophore (**3**) proceeded by straightforward extension of our model studies with **94**. A solution of the freshly purified aglycon **2** and the tris(triethylsilyl)-protected fucosyl α-trichloroacetimidate **93** (10 equiv) in deoxygenated benzene containing the Kishi free-radical inhibitor (2.5 mg/mL) was very briefly concentrated, and the residue was dissolved in ether. Addition of 3 Å molecular sieves and stirring at 23 °C (10 min) served to dry the solution; cooling to −30 °C and addition of trimethylsilyl triflate (0.1 equiv) then led to smooth α-fucosylation (63% yield, Scheme 18). Despite the instability of **2**, and the fact that some decomposition invariably occurred during its manipulation, with practice the procedure was reproducible and decomposition was minimal. It is interesting to note that the glycosylation reaction was apparently unaffected by the presence of the Kishi free-radical inhibitor,[48] which, however, helped to preserve the aglycon. The product (**101**) was more stable than the starting aglycon, but did readily decompose, especially when neat. Deprotection of the triethylsilyl ethers of **101** proceeded cleanly in 5% (v/v) hydrofluoric acid in acetonitrile at −30 °C, but did require a longer time for completion (1−1.5 h) than the model substrate (**97**). D-Fucosyl neocarzinostatin chromophore (**3**) was obtained in pure form after flash column chromatography (67% yield) and was found to be relatively stable toward concentration for brief periods. Like **1**, **3** was light-sensitive, and all operations handling it were conducted with the exclusion of light. Synthetic **3** provided spectral and analytical data fully consistent with expectations.

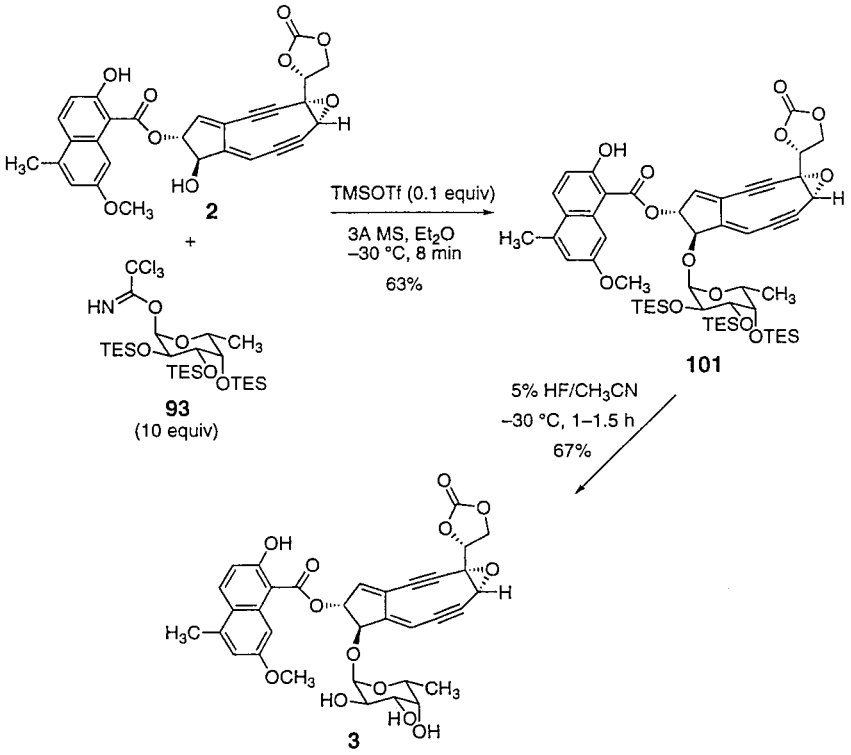
For further confirmation of structure, the product of nucleophilic activation with methyl thioglycolate (**102**, 47% yield, Scheme 19) was prepared. As in prior work with the aglycon itself (**2** → **90**), the latter reaction required the presence of an exogenous base (triethylamine, 150 equiv), this providing the most compelling evidence to date for the role of the methylamino group in thiol activation.[25,49]

Having established the suitability of **2** as a substrate for direct glycosylation, efforts turned toward the more challenging problem of introduction of the 2′-amino sugar of neocarzinostatin chromophore (**1**). Despite the importance of 2′-amino sugars in biology, reports of the stereocontrolled introduction of α-glycosidic bonds in 2′-amino sugars are few.[52] The key unresolved issue here was the nature of the protective group for the 2′-methylamino group. Although 2′-azido sugars (trichloroacetimidate activation) have been employed in α-selective glycosylation reactions,[53] conditions necessary for subsequent reduction of the 2′-azido substituent and *N*-methylation of the resultant 2′-amino group would almost certainly be incompatible with our target (**1**). Ideally, the group selected for protection of

(52) (a) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503. (b) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1380.

(53) (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.

Scheme 18



Scheme 19

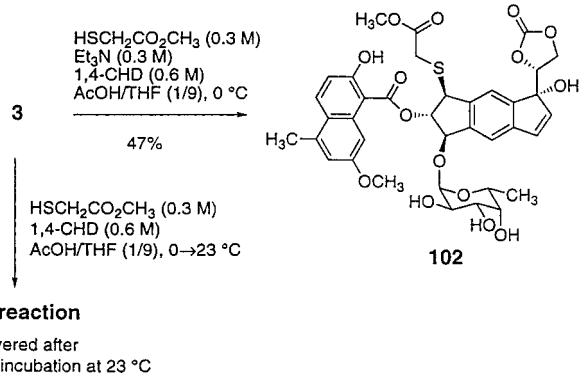
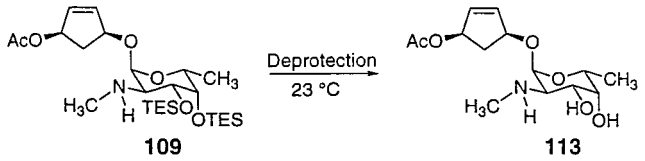


Table 1



conditions	result
10% HF, CH_3CN	30 min
**HF·pyr, THF	50 min
TBAF, AcOH	8 h
$Et_3N·3HF$, THF	no reaction
AcOH	no reaction

the 2′-methylamino group would be labile under the conditions of mildly acidic fluoride ion, identified as compatible with **1**. Acid-labile carbamates were reasonable to consider, but with them arose problematic issues in the glycosylation reaction. These are best illustrated by the results of our experiments with the *t*-Boc-protected 2′-deoxy 2′-methylamino α-fucosyl trichloroacetimidate **103** (Scheme 20). Neighboring-group participation of the carbamate during the glycosylation was evident from the *β*-selectivity of the reaction, and certainly was responsible for the formation of the oxazolidinone byproduct, a common liability of glycosyl donors with cation-stabilizing 2′-carbamate groups.[54]

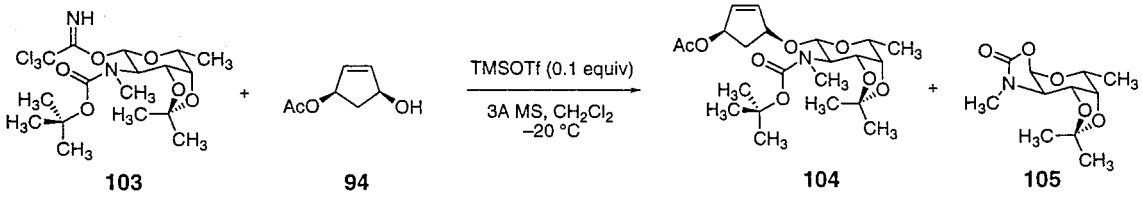
In a move away from carbamate protection of the 2′-methylamino group and toward the ultimately successful solution to the problem, we investigated the use of an acid-labile 2′-*N*-alkyl group, such as di-*p*-methoxyphenylmethyl (**106**, Scheme 21). In keeping with our earlier observations concerning bulky 2′-substituents, glycosylation with this substrate was found to be largely *β*-selective. The fact that a substrate with a nominally basic nitrogen atom was a viable glycosyl donor in the Schmidt coupling protocol, however, was revealing, and led us to question whether it was necessary to protect the 2′-methylamino group at all. To consider this question, the trichloroacetimidate **108**, with a free 2′-methylamino group and triethylsilyl ether protection of the two hydroxyl groups, was prepared and glycosylation of the model substrate **94** was studied.[24c,55] Coupling with **108** (Scheme 22) did require larger amounts of acid activator (up to 0.8 equiv, introduced portionwise) and longer reaction periods than substrates in which the 2′-methylamino group was protected, but **108** was nevertheless an excellent glycosyl donor. Optimum results were obtained by using triflic acid as activator (Scheme 22); under these conditions the coupled product (**109**) was obtained in 82% yield, with ≥20:1 α:*β* selectivity.

Mechanistically, this transformation is viewed to proceed via the 2′-methylamino-substituted oxocarbenium ion intermediate

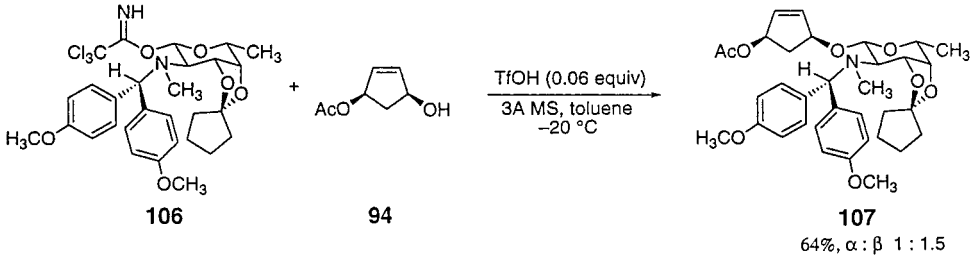
(54) Kuo, E. Y., Ph.D. Thesis, California Institute of Technology, 1993.

(55) 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.

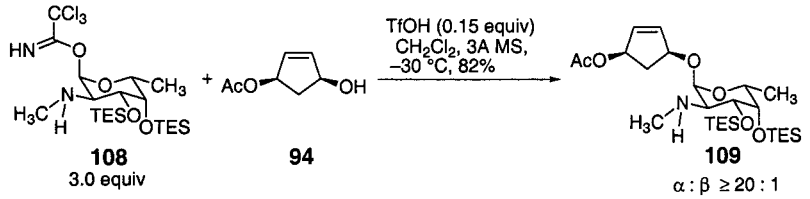
Scheme 20



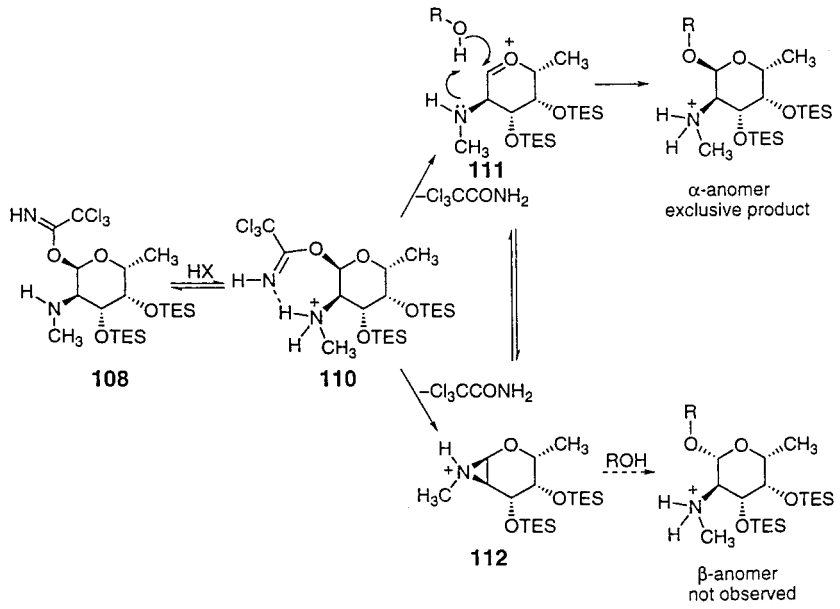
Scheme 21



Scheme 22



Scheme 23



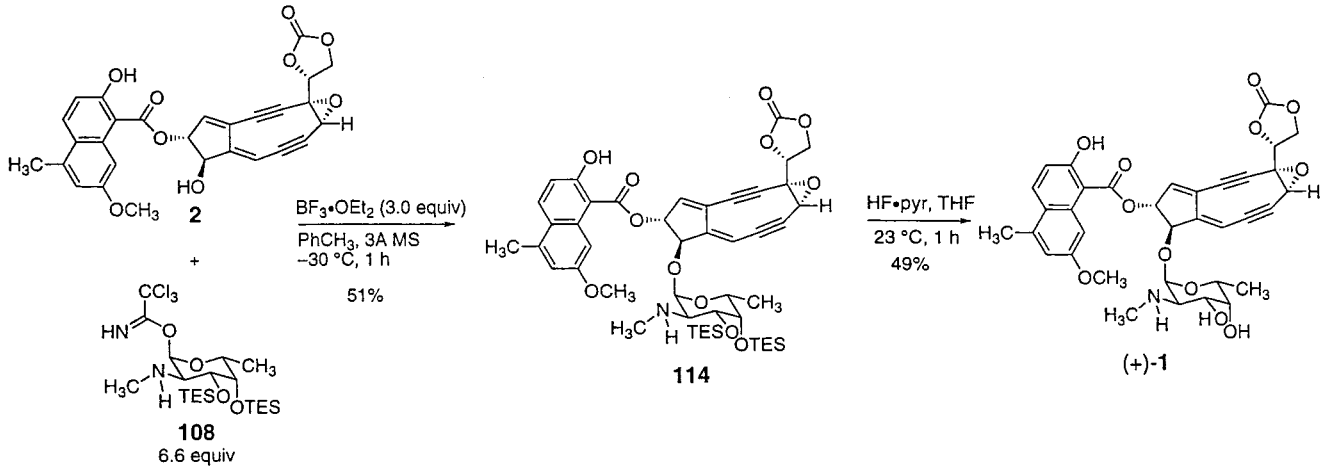
111, as outlined in Scheme 23. It is interesting to note that reaction via an *N*-methylaziridinium ion intermediate (**112**) would be expected to produce the isomeric β-product. Coupling under a wide variety of conditions was observed to be α-selective;[56] use of triflic acid as promoter, however, was optimal.

Conditions for the deprotection of the triethylsilyl ethers within the coupled product **109** were investigated (Table 1). We found that the presence of the free 2′-*N*-methylamino group in the substrate markedly slowed the rate of deprotection of the silyl ethers, but not to the extent that the synthesis of **1** by such a route became infeasible. In particular, hydrogen fluoride−pyridine complex provided a mild reagent for clean and rapid deprotection of **109**.

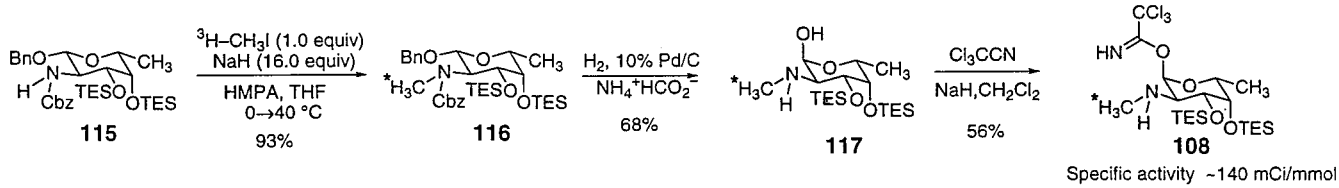
Extension of our model studies to the synthesis of **1** required the development of a slightly modified coupling protocol. After extensive experimentation, we found that the glycosylation of **2** with **108** (6.6 equiv) was best achieved by using boron fluoride etherate (3.0 equiv) as the Lewis acid promoter and toluene as

(56) Hirama's group has described studies of the neocarzinostatin chromophore glycosylation problem in which they employed a 2′-methylamino group in unprotected form. Their work involved thioglycoside activation with stoichiometric triflic acid, however, and gave rise to β-selective coupling. It is likely that the *N*-methylamino group was protonated in the transition state for this glycosylation reaction. Takahashi, K.; Tanaka, T.; Suzuki, T.; Hirama, M. *Tetrahedron* **1994**, *50*, 1327.

Scheme 24



Scheme 25



* indicates tritium label

the solvent (Scheme 24). Under these conditions, the glycosylation was complete within 1 h at −30 °C, affording the coupled product (**114**) in 51% yield after flash column chromatography on silica gel. The desired α-anomer was formed exclusively, as determined by ^{1}H NMR analysis. Because **114** was observed to decompose when stored (−80 °C, deoxygenated benzene matrix), deprotection was typically conducted immediately. Use of hydrogen fluoride−pyridine complex in THF cleanly removed both triethylsilyl ether groups within 1 h at 23 °C to afford synthetic neocarzinostatin chromophore (**1**) in unprotected form. Purification was achieved by direct loading of the deprotection reaction solution onto Sephadex LH-20 resin (attempted prior neutralization of the reaction medium induced rapid decomposition of **1**). With use of a mixed solvent system (dichloromethane, methanol, acetic acid; 94:5:1), pure **1** eluted from the column, while the lower molecular weight hydrogen fluoride−pyridine complex was retained. Synthetic **1** provided ^{1}H NMR, CD, and IR spectra that were identical with spectra obtained from a sample of natural chromophore, and both samples showed identical mobility in rp-HPLC analysis. We were also successful in obtaining a high-resolution mass spectrum of **1** using an external ion source Fourier transform ion cyclotron resonance (FT-ICR)[57] mass spectrometer equipped with a nanoelectrospray[58] ionization source (calcd for $C_{35}H_{34}NO_{12}$ ($[M + H]^+$), 660.2082; found, 660.2090). The exceedingly mild nature of this soft ionization technique was evident in the fact that the molecular ion peak was also the base peak; essentially no fragment-ion peaks were observed (<2% of base-peak intensity).

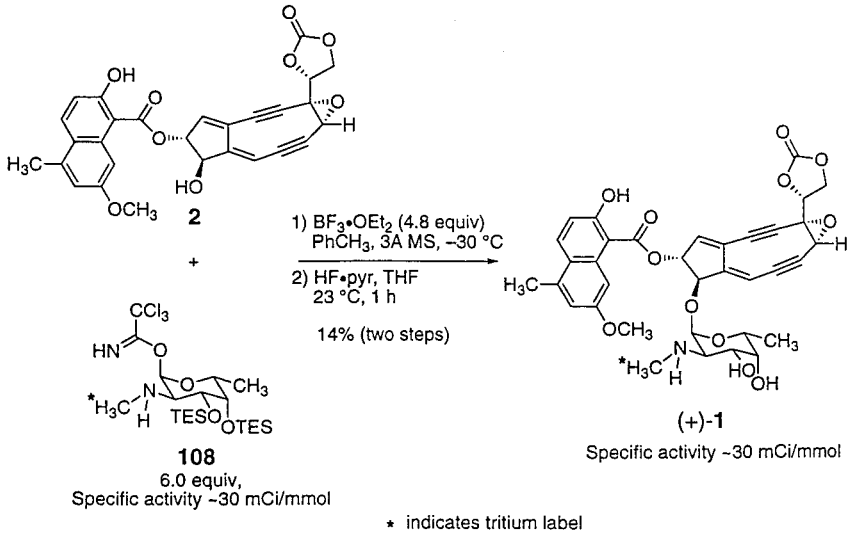
Synthesis of Singly and Doubly Radiolabeled Neocarzinostatin chromophore

The development of an efficient synthetic pathway to neocarzinostatin chromophore (**1**) provided a valuable opportunity to conduct a unique series of mechanistic studies, provided that the route could be modified to introduce one or more radiolabels within the molecule. The goal would then be to incubate a specific cell line with the labeled compound to determine the fate of the drug in vivo, certainly an unresolved issue at present. Here, we describe briefly only the chemical synthesis component of the problem and its solution as applied to the preparation of tritiated and ^{14}C-labeled **1**, both separately and in combination. For reasons of safety and economy it was desirable to incorporate the radiolabel as late in the synthetic route as possible. It was logical then that our first consideration was to label the glycosyl donor **108**; this was accomplished by using tritiated methyl iodide, as shown in Scheme 25. In the introduction of the radiolabel, the *N*-Cbz derivative **115** (used in excess) was deprotonated with sodium hydride in the presence of hexamethylphosphoramide (HMPA) and alkylated with ^{3}H-methyl iodide to afford the *N*-methylated product **116** in 93% yield (specific activity ∼140 mCi/mmol). Thereafter, the protocol for the synthesis of tritiated **1** followed established lines (Schemes 25 and 26). After purification by chromatography with Sephadex LH-20, the yield of synthetic ^{3}H-**1** was quantitated by measurement of the UV absorbance of the column fractions at 302 nm ($\epsilon = 5830\ M^{-1}cm^{-1}$); in a typical preparation, ∼0.4 mg ^{3}H-**1** was obtained, with a specific activity of ∼30 mCi/mmol.

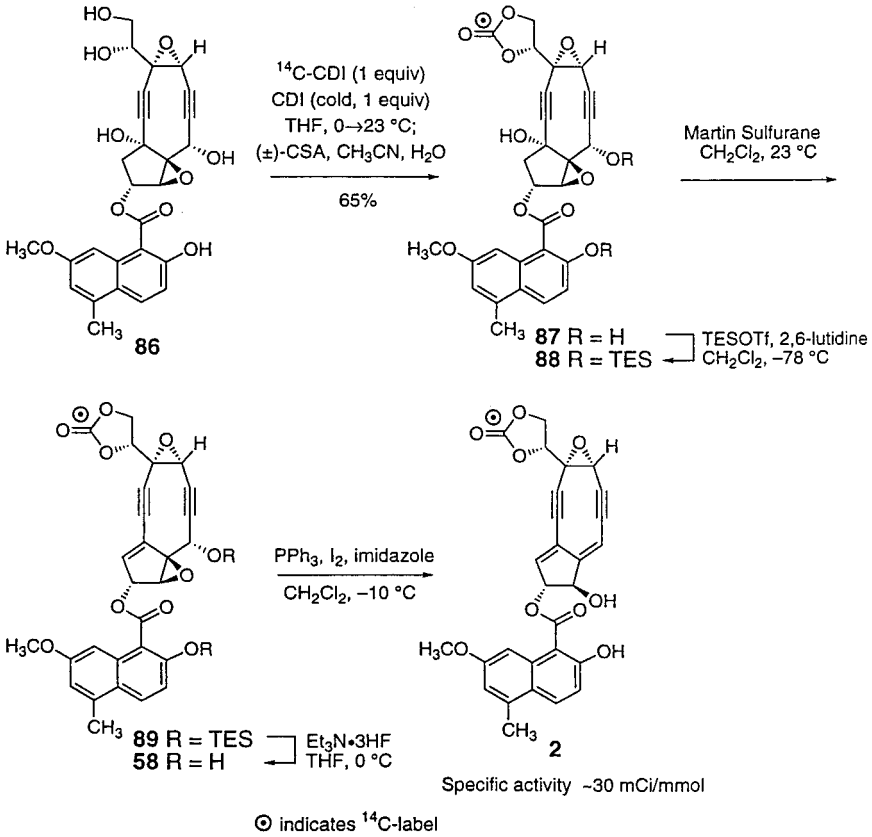
As in vivo mechanistic studies with ^{3}H-**1** progressed (detailed elsewhere), it became necessary to prepare **1** with an additional but different radiolabel, in a different part of the molecule. The ^{14}C-labeled aglycon **2** was therefore synthesized, as shown in Scheme 27. This route employed ^{14}C-labeled carbonyldiimidazole for introduction of the first radiolabel (**86** → **87**), and tritiated methyl iodide, as before, for the second label. In this manner, ∼0.4 mg ^{14}C-^{3}H-**1** was synthesized, with a specific

(57) Rodgers, M. T.; Campbell, S.; Marzluff, E. M.; Beauchamp, J. L. *Int. J. Mass Spectrom. Ion Processes* **1994**, *137*, 121.
(58) Wilm, M.; Mann, M. *Anal. Chem.* **1996**, *68*, 1.

Scheme 26



Scheme 27



^{14}C-radioactivity of ∼30 mCi/mmol and a specific ^{3}H-radioactivity of ∼30 mCi/mmol (Scheme 28).

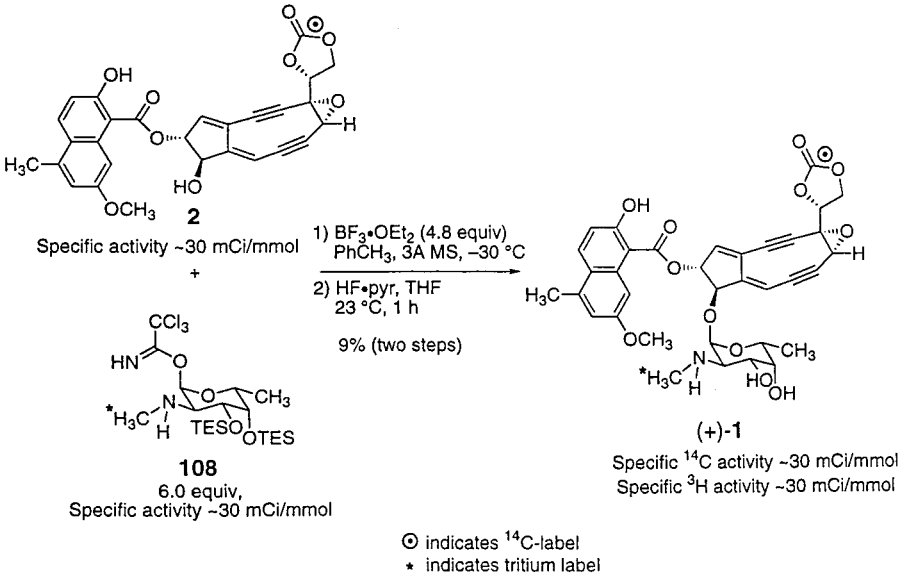
The ability to synthesize ^{3}H- and ^{14}C-^{3}H-labeled **1** by simple modification of our synthetic route is unique to that approach; it would have been difficult, if not impossible, to have prepared the same derivatives biosynthetically, for example.[59] The availability of these materials should enable the rather precise determination of the reaction pathways **1** follows in many different settings, in particular in vivo studies with different cancer cell lines.

(59) (a) Iwasaki, S. *Compr. Nat. Prod. Chem.* **1999**, *1*, 557. (b) Thorson, J. S.; Shen, B.; Whitwam, R. E.; Liu, W.; Li, Y.; Ahlert, J. *Bioorg. Chem.* **1999**, *27*, 172.

Summary

A convergent, enantioselective synthesis of neocarzinostatin chromophore (**1**) was developed. The previously unknown, and highly unstable aglycon (**2**) served as our key intermediate, synthesized in 19 steps (average yield per step 89%) from the three precursors (**13**, **14**, and **15**). It is noteworthy that the final step in the preparation (**58** → **2**, Scheme 16) was not a known transformation at the outset of our studies. This transformation allowed us to transition from a series of olefinic synthetic precursors to the corresponding epoxides, which proved to be markedly more stable and therefore more practical intermediates. Neither this stabilizing feature nor the success of the final

Scheme 28



transformation (**58** → **2**) could have been foreseen or predicted with any certainty.

Aglycon **2** was shown to be a viable substrate for glycosylation, despite its instability. The fucosyl (2′-methylamino→2′-hydroxyl) analogue **3** was thus prepared, using the Schmidt trichloroacetimidate protocol. This strategy was then extended to the preparation of **1**, with the key recognition that the 2′-methylamino group was best introduced without a protective group. Both glycosylation reactions were highly selective for the desired α-anomeric glycosides. Efficient procedures for the final deprotection and purification of synthetic **3** and **1** were also developed. To date, the route described has been executed successfully and independently by three different researchers for the preparation of milligram quantities of **1** and, as described, radiolabeled **1**. In addition to expanding our understanding of, and modes of implementation of, various basic chemical transformations (e.g., intramolecular acetylide additions, reductive transposition of 2,3-epoxy alcohols, glycosylation with 2-amino sugar donors), we believe that these studies will allow us to determine the fundamental chemistry of **1** that occurs in vivo.

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Supporting Information Available: Experimental procedures, schemes for synthesis of compounds **13**, **14**, **91**, **92**, and **93**, and characterization data for all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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