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Reactivity of Aziridinomitosene Derivatives Related to FK317 in the Presence of Protic Nucleophiles

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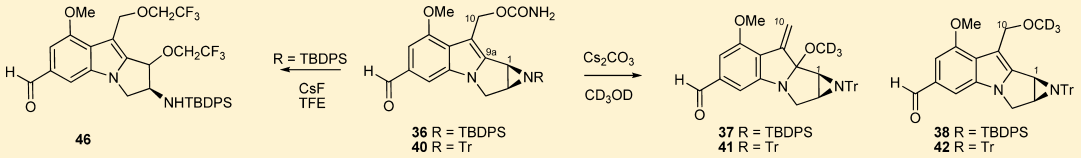
Supporting Information



ABSTRACT: The syntheses and reactivity of *N*-TBDPS and *N*-trityl protected derivatives of an aziridinomitosene corresponding to FK317 are described. New reactivity patterns were observed for these highly sensitive and functionally dense heterocycles under mild nucleophilic conditions approaching the threshold for degradation. Thus, the silyl or trityl protected aziridinomitosene reacted with Cs_2CO_3/CD_3OD to give isomeric products where substitution occurred at C(10) and C(9a) (mitomycin numbering) providing a CD_3 ether and a CD_3 hemiaminal, respectively. These findings show that heterolysis at C(10) is faster than at aziridine C(1), in contrast to the behavior of typical aziridinomitosenes in the mitomycin series. The labile *N*-TBDPS hemiaminal and the more stable *N*-trityl hemiaminal resemble the mitomycin K substitution pattern. A reagent consisting of CsF in CF_3CH_2OH/CH_3CN desilylated a simple *N*-TBDPS aziridine but caused nucleophilic cleavage at C(1) as well as C(10) without cleavage of the *N*-TBPDS group in the fully functionalized penultimate aziridinomitosene. The high reactivity of the C(10) carbamate with nucleophiles precludes the use of deprotection methodology that requires *N*-protonation for fully functionalized aziridinomitosenes in the FK317 series.

INTRODUCTION

The FR family of anticancer prodrugs **1**−**4** is structurally related to mitomycin C **6** (Figure 1)[1−4] and has a similar requirement for reductive conversion into an active form that is responsible for DNA cross-linking at the 5′CG 3′ sequence.[5,6] Fukuyama proposed that the active form is the labile aziridinomitosene **5** (R = Y = X = H) resulting from reductive N−O bond cleavage to a benzazocinone core followed by cyclization and loss of water.[7] Although detection of the labile azirdinomitosene has not been reported, the DNA lesion expected from activation of FR66979 (**2**) and DNA alkylation by the C(1) and C(10) positions of **5** has been characterized.[8] Members of the FR family were reported to have better activity and lower toxicity compared to the mitomycins,[4,9,10] and the semisynthetic **4** (FK317), a diacetate analogue of **1**, was identified as having a promising activity profile with decreased incidence of vascular leak syndrome compared to **1**.[4b] These developments have stimulated extensive studies that establish access to **1**−**3** by total synthesis,[11,12] but relatively little is known regarding the corresponding activated intermediates related to the aziridinomitosenes **5**. Given the exceptional solvolytic reactivity of **5** in the mitomycin series, it remains to be seen whether any FR- or FK-derived analogue of **5** (Y = X = H) is stable enough to prepare by synthetic means.

Earlier reports from our laboratory described a synthetic approach to structures **7** and **8**, aziridinomitosene analogues that are related to the hypothetical activated form **9** corresponding to FK317 (**4**) (Scheme 1).[13−15] Selective deprotection of **8** by cleavage of the *N*-trityl group using reductive detritylation conditions (i-Pr_3SiH/MsOH)[16] gave the N−H aziridine **7** in 42% yield. However, attempted detritylation of the late stage intermediate **10**, containing the correct C(9) carbamate functionality, was not possible due to competing heterolysis and reductive trapping at C(10).[15] The greater sensitivity of **10** vs **7** or **8** reflects increased electron density in the indole ring, an effect that is expected to promote heterolysis at C(10) as well as C(1) in the absence of the stabilizing C(9) ester group. We now report further exploration of **7** and derivatives as part of our efforts to probe the chemistry of labile aziridinomitosenes and to carry out late stage synthetic transformations in the highly sensitive, fully functionalized environment. These new studies help to better define the competing pathways in reactions at the C(1), C(9a), and C(10) positions with nucleophiles.

RESULTS

Key substrates were prepared using the previously reported convergent sequence via alkylation of a protected indole ester **11**[15] with the mesylate **13** derived from tributylstannylaziridine alcohol **12**[14] to afford **14** (Scheme 1). Introduction of a deuterium blocking group (**15**) to prevent competing indole lithiation (**16**) followed by tin−lithium exchange resulted in intramolecular 1,4-addition to form the enolate **17**. Subsequent selenenylation/oxidative elimination afforded tetracycle **18**, functional group manipulations provided **8**, and reductive

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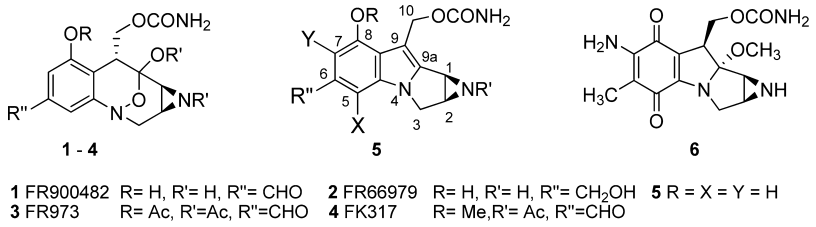
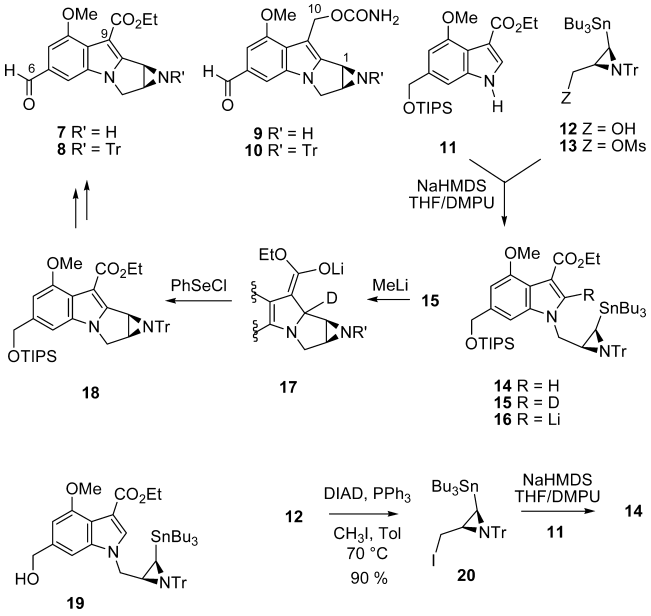


Figure 1. Natural and semisynthetic precursors of aziridinomitosenes **5**.

Scheme 1

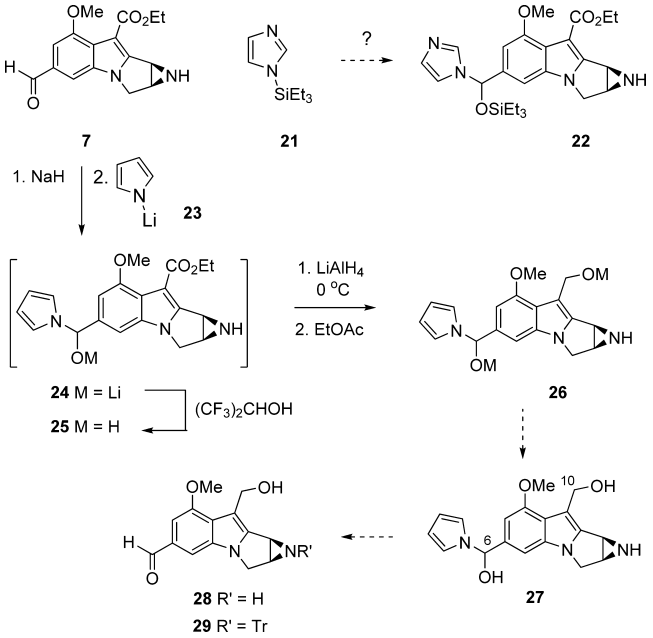


detritylation[16] gave the N−H aziridine **7** following the published route with small adjustments in late stage procedures (see Supporting Information). The technically challenging conversion from **14** to **18** was carried out in acceptable 75% yield, but the *N*-alkylation from the aziridine **13** to **14** proved more troublesome and the high yields reported previously could not be reproduced. Typical experiments produced **14** in 54% yield after 4 d at 60 °C with 3 equiv of **11** and 3 equiv of NaHMDS in 2:1 THF: DMPU, along with 17% of the undesired desilylated alcohol **19**. Repeated attempts failed to identify or suppress the nucleophile responsible for the premature desilylation, so the options for the conversion from **11** to **14** were re-evaluated. More consistent results were obtained in the *N*-alkylation when mesylate **13** was replaced by the iodide **20**, available in excellent yield from **12** using a Mitsunobu procedure.[17,18] This gave **14** in 65% isolated yield based on the iodide **20** and only traces of **19**, and the reaction time was considerably shorter (3 h) compared to the mesylate reaction (4 d).

Most samples of the *N*-H aziridinomitosene **7** prepared in the current study were found to consist of two aziridine invertomers according to NMR evidence,[16] in contrast to the single species observed for the material isolated previously. Thus, two pairs of broad aziridine methine signals appeared between ^{1}H δ 3 and 4 ppm, and a coupling constant of 4.2 Hz was extracted for one of the invertomers by homonuclear decoupling and D_2O exchange experiments. This coupling is small for a *cis* aziridine, but it is consistent with the spectra of other aziridinomitosenes.[18−20] Coalescence into a single pair of aziridine methine signals at 4.04 and 3.60 ppm was observed upon warming the sample to ca. 45 °C. The variable NMR spectra for the *N*-H aziridinomitosene **7** reflect the presence of nitrogen invertomers. The structure of **7** was confirmed by treating the material with trityl chloride (1 equiv) and Hünig's base (2.2 equiv; CH_2Cl_2, 0 °C to rt) to regenerate **8** (34% recovered). The N−H aziridine **7** is more susceptible to decomposition than **8** due to removal of the bulky trityl group, but purified **7** can be stored at low temperature for extended periods.

Early stage aziridine deprotection complicates handling of products during and after the selective reduction at ester C(10). This conversion is challenging under the best of circumstances because the resulting primary alcohol at C(10) is highly sensitive under acidic or neutral protic conditions in the absence of the electron-withdrawing C(10) ester group that helps to prevent aziridine heterolysis. Another complication is the presence of the C(6) formyl group. Temporary protection at C(6) is essential during ester reduction, but masking the C(6) formyl also temporarily cancels its stabilizing electronic effect and contributes to the solvolytic reactivity of the product. Therefore, the aldehyde protecting group must be removed using basic conditions immediately after reduction, but prior to product isolation. In principle, this approach restores the stabilizing effect of the formyl group and improves chances for product survival during purification. The initial plan was to mask the C(6) aldehyde using *N*-trimethylsilylimidazole **21** (Scheme 2), following a procedure that had been developed

Scheme 2



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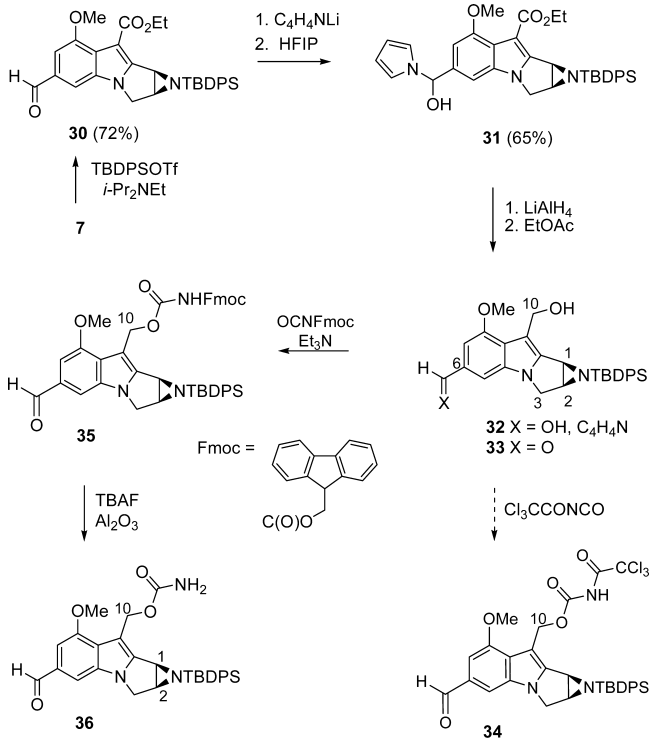
from the *N*-trityl aziridine **8**.[15] However, numerous attempts to perform the conversion from **7** to **22** failed to reach an acceptable level of conversion according to NMR assay. Furthermore, crude **22** proved to be surprisingly labile compared to the *N*-trityl analogue **8**. Together with the substantial increase in polarity, the stability problem frustrated all attempts to purify **22**. Finally, the presence of decomposition products as well as presumed aziridine invertomers in **22** resulted in complex spectra, and left the outcome of these experiments open to question.

As an alternative to **22**, the base-labile pyrrole hemiaminal strategy developed by Evans[21] and Dixon[22] was investigated for the purpose of masking the formyl group. Not surprisingly, installation of the pyrrole on tetracycle **7** also proved to be difficult. Attempted addition of C_4H_4NLi followed by workup with proton donors such as water or methanol always returned mixtures containing mostly recovered **7** as well as the pyrrole hemiaminal. Evidently, the basic medium resulted in equilibrium between the desired **25** and the alkoxide **24**, and promoted cleavage of the adduct back to the starting material **7**. To avoid the equilibrium problem, as well as to minimize potential risks of acid-induced aziridine cleavage, a nonaqueous quenching procedure was developed using hexafluoroisopropyl alcohol (HFIP)[23] as the proton donor (1:2 of C_4H_4NLi/HFIP). This procedure improved conversion and allowed the isolation of **25**, but recovery of the starting **7** was still problematic. Finally, it was found that adding one equiv of NaH to **7** prior to addition of excess C_4H_4NLi provided **25** in improved 63% yield. The role of the NaH is not known, but temporary deprotonation of the potentially troublesome aziridine N−H is one possibility. Once purified, the pyrrole hemiaminal **25** was reasonably stable if stored in the freezer.

The stage was now set for the challenging reduction of the ester **25** with $LiAlH_4$. Selective reduction requires that the intermediate alkoxide **26** does not dissociate to the aldehyde in the presence of the reducing agent. Furthermore, isolation of the primary alcohol **27** with the pyrrole hemiaminal intact at C(6) increases the risk of C(1)-N or C(10)-O heterolysis due to the absence of the stabilizing π acceptor as already mentioned. With these issues in mind, **25** was treated with excess $LiAlH_4$ at 0 °C[13] and the reaction was quenched with ethyl acetate containing trace dissolved water, thereby avoiding the risky aqueous workup. The ethoxide generated during reduction of the ethyl acetate facilitated cleavage of the pyrrole hemiaminal **27** in one pot to regenerate the C(6) formyl group (**28**). Filtration through Celite with MeOH provided a crude product that was difficult to assay due to poor solubility in organic solvents, but two interesting characteristics could be observed: a formyl proton at δ = 9.9 ppm in the ^{1}H NMR spectrum (CD_3OD), and a broad IR stretch at 3450 cm^{-1}. This information suggests successful reduction at C(10), subsequent unmasking at C(6) and formation of **28**. However, the C(10) methylene and aziridine protons were obscured by other signals and could not be observed, and the product could not be purified or further characterized due to its limited quantity, high polarity, and insolubility. Therefore, installation of the C(10) carbamate at the stage of **28** was not attempted and an alternative strategy was evaluated.

Plans were adjusted to exploit the apparent correlation between better stability and solubility observed for aziridinomitosenes containing the bulky, hydrophobic *N*-trityl group (**9** or **29**) compared to N−H aziridines (**7** or **28**) by replacing the *N*-trityl group with a bulky *N*-silyl group.[24] In principle, desilylation might be possible using nucleophilic "silaphiles" without resorting to acidic conditions that destroy the sensitive aziridinomitosene core.[19,25−27] Accordingly, the *t*-butyldiphenylsilyl (TBDPS) group was installed by treating **7** with 1 equiv of freshly prepared TBDPS triflate (TBDPSOTf)[28] and Hünig's base to provide **30** (Scheme 3) after workup with a basic buffer

Scheme 3. TBDPS-Protected Aziridinomitosenes



solution (pH 10). A similar experiment using TIPSOTf/Hünig's base did not give acceptable conversion, and the *N*-TIPS aziridinomitosene was not sufficiently stable for purification. In contrast, **30** survived Et_3N-deactivated silica gel chromatography, could be stored at −20 °C in frozen benzene, and was sufficiently stable for the next stages.

Attention was turned to masking the aldehyde group of **30** by treatment with *N*-lithiopyrrolidine to give the pyrrole hemiaminal **31** (Scheme 3). In addition to the risk of C(1) and C(10) heterolysis, the conversion to **31** must also deal with the potential problem of premature *N*-desilylation. Indeed, aqueous workup led to mixtures of **7**, **30** and **31**. Fortunately, the nonaqueous workup using hexafluoroisopropyl alcohol (HFIP)/ether quenching allowed recovery of pyrrole hemiaminal **31** in >60% yield. Although **31** could be separated from impurities on deactivated silica gel, solvent removal or storage at −20 °C resulted in unmasking of the aldehyde and decomposition. In particular, attempts to remove the last traces of Et_3N and residual water obtained by elution of the buffered silica gel resulted in time-dependent decomposition. Similar problems was encountered in all subsequent stages of this investigation, and were never fully controlled, although limiting the time scale for solvent removal allowed recording informative NMR spectra of material containing residual Et_3N, water, and traces of elution solvent. In the best isolation experiment, **31** was obtained along with 6% of aldehyde **30**, but it was necessary to perform subsequent operations on material

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immediately after chromatography without allowing time for removal of the last traces of elution solvents.

The reduction of **31** to **32** must contend with the risk of *N*-desilylation, the ever present hazards of heterolysis at C(10)−O and C(1)−N, the polarity of **32**, and the challenge of avoiding reduction at C(6). On the other hand, polarity should be lower and stability should be improved at the aldehyde stage (**33**) while the *N*-TBDPS group should facilitate recovery compared to the N−H analogue **28**. In the event, freshly chromatographed **31** was treated with 5 mol equiv of $LiAlH_4$ at 0 °C followed by quenching as described starting from **25**. In preliminary experiments, two major products were detected by ^{1}H NMR spectroscopy (ca. 1:1 ratio), one of which was stable enough to isolate and was assigned as the aldehyde **33** based on the characteristic signal at δ 9.95 ppm. Aldehyde **33** also showed an ABX pattern (δ 4.95 and 4.76 ppm) coupled to a doublet of doublets (δ 2.88 ppm), assigned as the C(10) methylene protons and the hydroxyl proton, respectively (Table 1, entry 4). The distinctive C(1) and C(2) aziridine protons were also present, but the C(1) doublet moved upfield from δ = 3.62 ppm (**30**) to δ = 3.29 ppm (**33**), thereby confirming a change in oxidation state at C(10) (Table 1, entries 1 and 4). Like the ester **31**, aldehyde **33** survived rapid chromatography, but decomposed during prolonged (>1d) storage in frozen benzene. The other product from reduction was too sensitive to isolate, but was assigned as **32** due to the similarity of the upfield ^{1}H NMR signals to those of **33** (Table 1, entry 3), plus signals consistent with the presence of the pyrrole moiety. Fortunately, the undesired recovery of **32** could be avoided by increasing the LAH quenching time with ethyl acetate to ca. 1.5 h at rt, resulting in a 14:1 ratio of **33**:**32** in the best case.

Table 1. Characteristic ^{1}H NMR Signals of 30−36[a]

entry	tetracycle	C(1)	C(2)	C(3)	C(10)
1	**30**	3.62 d	3.38 dd	4.27 d	
				4.07 dd	
2	**31**	3.57 d	3.31 d	4.17 d	
				3.95 dd	
3	**32**	3.22 d	3.28 dd	obsc	4.93 dd
					4.73 dd
4	**33**	3.29 d	3.6 dd	4.21 d	4.95 dd
				4.03 dd	4.76 dd
5	**34**	3.53 d	obsc	obsc	5.66 d
					5.56 d
6	**35**	3.36 d	3.29 dd	obsc	5.65 d
					5.20 d
7	**36**	3.41 d	3.36 dd	4.23 d	5.47 ABq
				4.02 dd	

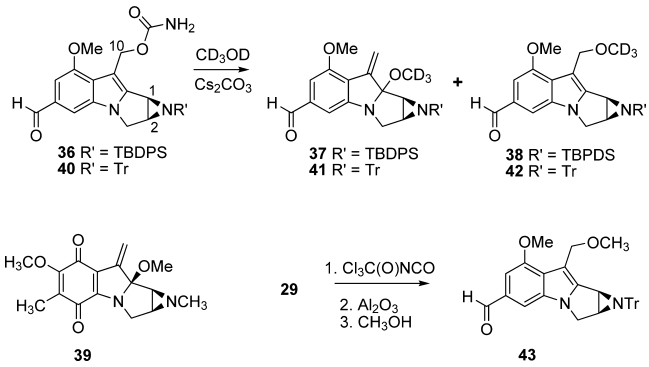
[a]Chemical shifts in ppm.

The inherent risk of heterolysis at the stage of **33** severely restricts the options for carbamoylation at C(10) (Scheme 3). Following precedents,[29,30] **33** was treated with 1 equiv of trichloroacetyl isocyanate between −78 °C and rt for 1 h to give material having ^{1}H NMR signals consistent with the expected C(10) methylene AB pattern of **34** (δ ca. 5.6 ppm; Table 1, entry 5). However, attempted cleavage of the trichloroacetyl group from the presumed **34** with K_2CO_3 in MeOH[29] resulted in disappearance of the C(10) signal, but produced no signals consistent with simple cleavage of the trichloroacetyl group, nor with recovery of **33**. Therefore, an alternative method for carbamate installation using the milder FmocNCO reagent was investigated.[18,31] Initially, **33** was treated with freshly prepared FmocNCO/Et_3N to give a crude product having characteristic C(10) methylene signals near 5 ppm, suggesting formation of **35**. Attempts to remove the Fmoc group from the presumed **35** using previously optimized conditions (Et_3N/CH_3CN) resulted in significant decomposition.[29] However, treatment of **33** with FmocNCO/Et_3N followed by excess TBAF on alumina provided the free carbamate **36** as a white solid after filtration and solvent removal.[32,33] Although this selective cleavage was welcome, it was rather sobering. We had imagined that the fluoride reagent might also cleave the previously labile *N*-TBDPS group, an event that would solve all of the deprotection problems in one operation, but this was not to be. The ^{1}H NMR spectrum clearly showed the characteristic TBDPS signals as well as the C(1) and C(2) methine signals of an intact, N-silylated aziridine (Table 1, entry 7).

Treatment of **36** with anhydrous CsF or KHF_2 in THF at rt did not effect desilylation, while Et_3N-HF and TAS-F resulted in decomposition.[34] This initially surprising resistance to fluoride donors stimulated an experiment to confirm that the *N*-TBDPS group is sensitive to protic conditions, as noted in connection with attempts to prepare ester **30**. Thus, **30** was dissolved in CD_3OD and monitored by ^{1}H NMR spectroscopy over 20 h. During this time, the signals of N−H aziridine **7** slowly appeared, along with a new TBDPS signal at δ 1.01 ppm, as expected from attack of nucleophilic deuterated methanol at the silicon to form $TBDPSOCD_3$. In the hope that these conditions would be mild enough, aziridinomitosene **36** was dissolved in CD_3OD and the sample was monitored by ^{1}H NMR spectroscopy. The expected $TBDPSOCD_3$ signal at δ = 1.01 ppm appeared within 45 min, but new C(1) and C(2) methine signals corresponding to the deprotected N−H aziridinomitosene were never observed. Instead, the ^{1}H NMR spectrum showed complex new signals between 3.8 and 4.2 ppm, consistent with the chemical shift range for products of aziridine ring cleavage.[26b]

Since the undesired aziridine C(1) heterolysis implies *N*-protonation as the activating event, the methanolysis experiment was repeated in the presence of a base in the hope that nucleophilic attack at Si may become competitive (Scheme 4).

Scheme 4



Thus, carbamate **36** and Cs_2CO_3 were dissolved in deuterated methanol, and the reaction was monitored by ^{1}H NMR spectroscopy over a 10 h period. No evidence was found for the desired silyl group cleavage. Instead, the NMR spectrum indicated that tetracycle **36** had undergone surprising structural

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changes. Multiple new peaks appeared while the signals corresponding to **36** decreased in intensity. Two new products were observed after 17 h at rt in a 1:1 ratio, but after 6 d only one of these products (**37**) was present. According to the ^{1}H NMR spectrum, the aziridine C(1) and C(2) signals as well as the C(3) methylene signal of the more stable product had moved upfield compared to carbamate **36** (compare Table 2, entry 1, with Table 1, entry 7). More surprisingly, the C(10) AB quartet was missing, but two vinylic protons at δ = 5.54 and 6.28 ppm were present. The nominal mass (ESMS; m/z = 514 amu) corresponded to loss of the carbamate and incorporation of OCD_3. Based on the above data and comparison with the natural product mitomycin K (**39**; Table 2, entry 3),[35] the exocyclic olefin structure **37** of the stable product was tentatively assigned. Further support for the structure by ^{13}C NMR was precluded by the small quantity obtained and the inability to completely purify **37**.

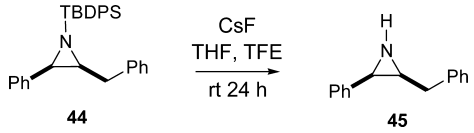
Table 2. Characteristic ^{1}H NMR Signals of Key Tetracycles[a]

entry	tetracycle	C(1)	C(2)	C(3)	C(10)
1	**37**	2.66 d	2.43 dd	3.55 d	6.28 d
				3.34 dd	5.54 d
2	**38**[b]	3.41 d	3.53 dd	obsc	4.79 ABq
3	**39**[35]	2.25 s	2.25 s	4.08 d	6.32 s
				3.41 d	5.50 s
4	**41**	2.28 d	2.13 dd	3.80 d	6.29 d
				3.40 dd	5.47 d
5	**42**	3.04 d	3.01 dd	4.44 d	4.89 d
				4.10 dd	4.73 d

[a]Chemical shifts in ppm, $CDCl_3$. [b]CD_3OD solution

To support the structure of **37** by analogy and to better understand the methanolysis process, the relatively well-behaved *N*-Tr aziridinomitosene **40**[15] (Scheme 4) was exposed to the basic solvolysis conditions (Cs_2CO_3 in deuterated methanol, monitoring by ^{1}H NMR spectroscopy). Analogous changes appeared in the NMR spectrum as observed starting with **36**, and two major products were observed in a 1:1 ratio after 3 d. One of the products (**41**) correlated well with mitomycin K (**39**) and also with **37** in terms of ^{1}H NMR chemical shifts (Table 2, entry 4). Furthermore, the presence of two alkenyl carbons (δ 140.2 and 114.4 ppm) and a quaternary hemiaminal carbon (δ 106.1 ppm) were confirmed by ^{13}C NMR spectroscopy. The second product was also isolated, and the molecular ion observed by ESMS indicated loss of the C(10) carbamate and incorporation of OCD_3 (M + Na, m/z 540 amu). Inspection of the ^{1}H NMR spectrum showed a pair of AB doublets at δ = 4.89 and 4.73 ppm, similar to C(10)−O derivatives such as the alcohol **32** (Table 1, entry 3). Although the other signals were initially puzzling, they proved to be identical to signals of the methyl ether **43** (unexpectedly obtained from alcohol **29** among other products after treatment with trichloroacetyl isocyanate, followed by alumina and quenching with methanol), excepting the absence of the singlet of the C(10) methoxy group (Table 2, entry 5). Thus, the "unknown" product is **42**, and differs from **43** only by the presence of OCD_3 in place of OCH_3. By analogy, the transient compound observed upon reaction of **36** with Cs_2CO_3 in deuterated methanol was assigned as **38**. Although the contrasting outcome of methanolysis in the presence or absence of carbonate with these functionally dense heterocycles is intriguing in the context of DNA cross-link formation, the susceptibility of the carbamate **36** to nucleophilic attack at C(9a) as well as C(10) severely limits the options for selective removal of the TBDPS group in the sensitive environment.

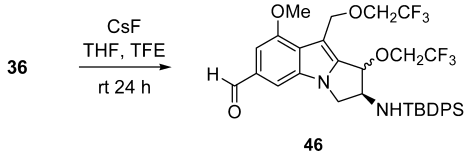
In an attempt to better understand, and hopefully to improve, the *N*-desilylation process using fluoride sources, a model aziridine **44** was briefly investigated (eq 1). Treatment of



44 **45** (1)

44 with commercial TBAF/DMF gave **45** (56% after 3 h at rt),[36] presumably because residual water[37] in TBAF acts as the source of the N−H proton. In contrast, the nonhygroscopic, crystalline tetrabutylammonium difluorotriphenylsilicate (TBAT)[38] did not desilylate **44** in deuterated DMF even after 3 d (^{1}H NMR assay). However, when the TBAT experiment was repeated in the presence of 1 equiv of trifluoroethanol as a stoichiometric proton source,[23] desilylation to **45** did occur (75% conversion in 24 h) and a similar experiment with CsF as the fluoride donor resulted in 95% conversion within 20 h. A simpler procedure using CsF in 2,2,2-trifluoroethanol (TFE) solution was also tested, and returned 61% of the desilylated aziridine **45** on preparative scale (2 d reaction time). However, only 14% desilylation was observed in trifluoroethanol without any added fluoride under similar conditions in an NMR experiment. These observations suggest that a proton source is necessary to activate aziridine nitrogen for N−Si cleavage by nucleophilic attack, and that trifluoroethanol is not sufficiently nucleophilic for a convenient rate of desilylation in the absence of fluoride. Thus, the combination of an anhydrous fluoride source with the relatively non-nucleophilic trifluoroethanol offered some hope that desilylation of the sensitive aziridinomitosene **36** might be faster than heterolysis at C(10). However, the same nitrogen activation event (presumably, *N*-protonation) required for desilylation might also promote heterolysis at C(1).

Aziridinomitosene **36** was subjected to the reoptimized desilylation conditions (1 equiv of CsF in 8:1TFE:THF), but no reaction was observed after 2 h. Conversion did occur after 1 d in the presence of excess CsF, and the major product diastereomer detected by ^{1}H NMR spectroscopy was stable enough for chromatographic isolation (eq 2). A second



46 (2)

diastereomer was also detected. Although it could not be fully separated, meaningful NMR data were obtained. Major changes were evident in the NMR spectra of both diastereomers compared to the starting **36**, and it was clear that the *N*-TBDPS group had survived the CsF/TFE treatment while the aziridine ring had not. Thus, both products showed a new doublet near δ = 4.6 ppm, coupled to a multiplet near δ = 3.9 ppm, consistent with aziridine ring cleavage by attack at C(1) involving an oxygen nucleophile.[26b] Also, the C(10) methylene protons of both products had moved upfield from δ 5.4−5.5 to ca. 5 ppm, suggesting that the carbamate had been replaced by an alkoxy group. Together with molecular ion data

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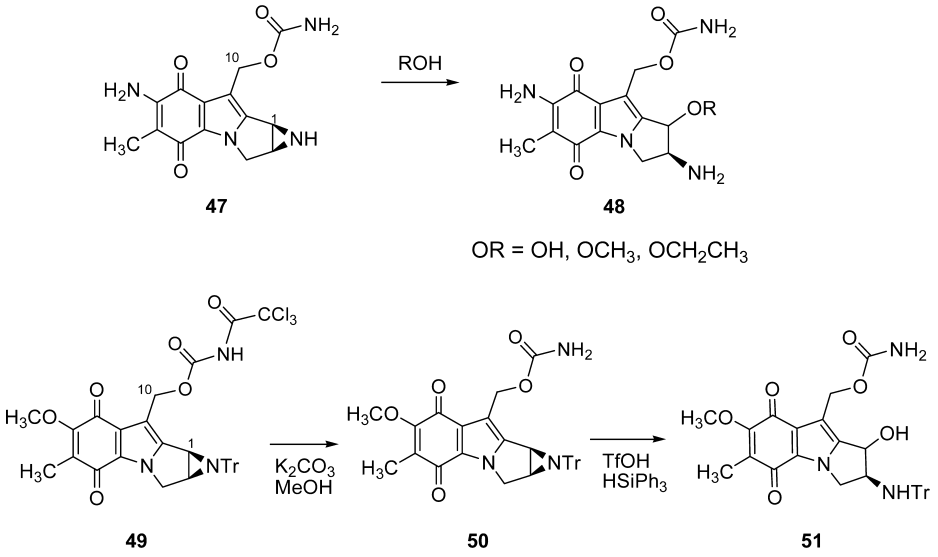
Scheme 5. Solvolysis of Aziridinomitosenes



corresponding to incorporation of 2 equiv of trifluoroethanol (ESMS, M + Na, m/z = 701 amu), the products were assigned as diastereomers of the ring opened mitosene **46**. Further support for this structure was provided by the observation of two triplets in the ^{19}F NMR spectrum for each diastereomer, and by homonuclear and heteronuclear (^{19}F) decoupling experiments. Although the incorporation of 2 equiv of the non-nucleophilic TFE was not desired, more concerning was the survival of the *N*-TBDPS protecting group on the nitrogen. Clearly, nitrogen activation had occurred, and just as clearly, heterolysis at C(1) was faster than nucleophilic attack at silicon. The aziridinomitosene **36** had proven to be sensitive to nucleophilic attack at both C(1) and C(10) under the mildly acidic protic conditions, and undesired heterolysis had taken place faster than desilylation.

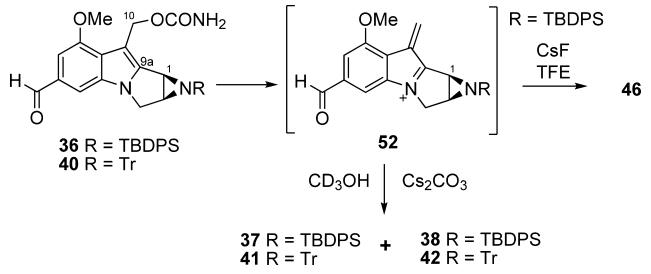
DISCUSSION

Extensive prior literature indicates that the C(10) carbamate of mitomycin C (**6**) is generally less reactive than the C(1) aziridine, although there are several competing pathways and both C(10) and C(1) are involved in the DNA cross-linking mechanism under conditions of reductive activation.[39] Under modified conditions, monoalkylation of DNA by mitomycin C is also known where alkylation occurs at C(1) but not at C(10).[40] Kohn has shown that the key aziridinomitosene C (**47**) is an intermediate in one of the activation pathways, and that preformed **47** monoalkylates DNA.[41] In the presence of exogenous nucleophiles, **47** reacts at C(1) and not C(10) to afford diastereomeric substitution products (Scheme 5).[19,26b]

Similar regioselectivity has been observed with a synthetic quinone derivative of aziridinomitosene A (**50**), generated by methanolysis of **49**.[29] Thus, treatment of **50** under reductive detritylation conditions gave **51** by heterolysis and nucleophilic attack at C(1). In contrast to **36**, the C(10) carbamate of **50** survived the K_2CO_3/MeOH procedure as well as the acidic conditions in the attempted detritylation.[29] The lower reactivity at C(10) vs C(1) in the quinones **47**, **49**, and **50** reflects the influence of delocalization involving the stabilizing quinone carbonyls. Only one prior investigation has explored the solvolytic behavior of a well-defined synthetic substrate that does not contain the stabilizing quinone carbonyls. Thus, Egbertson and Danishefsky reported that treatment of the hydroquinone corresponding to *N*-methylaziridinomitosene A with potassium ethyl xanthate in aqueous pyridine affords two nucleophilic substitution products, one resulting from attack at C(1) as well as C(10) (5%), and the other from attack exclusively at C(10) (8%).[27b] This latter product is noteworthy because nucleophilic substitution in the fully aromatic substrate occurs at C(10) without prior attack at aziridinomitosene C(1), in contrast to virtually all of the literature reports describing reductive activation of mitomycins.

In the FK317 series, structures such as **36** or **40** are also fully aromatic. Although they are presumably stabilized somewhat by the electron-withdrawing C(6) formyl carbonyl, the effect should be small compared to that of the mitomycin quinone carbonyls. Reactivity in **36** or **40** is dominated by the donor indole nitrogen, and the activating effect is felt most strongly at C(10). The first indications of this regioselectivity were seen earlier when attempted reductive detritylation of **40** with MsOH/triisopropyl silane gave a product of hydride capture at C(10).[15] In the current study, the same preference for C(10) heterolysis has been demonstrated in the methanolysis of **36** and **40**. Heterolysis at C(10) was also seen upon CsF/trifluoroethanol treatment of **36**. Selective attack at C(10) can be understood by invoking the formation of an iminium ion **52** starting from the FK317 derivatives **36** or **40** as the first step in the heterolysis sequence (Scheme 6). Although these

Scheme 6. Heterolysis of C(10) Carbamates 36 and 40



observations are directly relevant only to several transformations conducted in organic solvents, they do raise the possibility that the first heterolytic event in DNA alkylation by

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aziridinomitosene intermediates derived from FK317[42] and related structures also involves alkylation at C(10) and not at C(1).

SUMMARY

The fully functionalized N−H aziridinomitosene of FK317 **4** remains an elusive target. Selective removal of the *N*-TBDPS group could not be accomplished at the stage of **36**. Based on model studies as well as observations made with the substrates **36** and **40**, protonation at aziridine nitrogen is a prerequisite for desilylation as well as for detritylation. At this stage of our understanding, deprotection strategies that rely on activation by aziridine *N*-protonation are plausible only for aziridinomitosenes that contain stabilizing conjugated electron-withdrawing substituents, as in the methanolysis from *N*-TBDPS aziridine **30** to the N−H aziridine **7**. Although it has not been possible so far to detect hypersensitive aziridinomitosene analogues **5** that lack stabilizing substituents, the survival of **36**−**38** and **40**−**42** under carefully controlled conditions suggests that spontaneous heterolysis at C(1) is not a major hazard, contrary to our expectations. Stability of the C(10)−O bond is more problematic, although detection (and in some cases, isolation) of structures such as **29**, **35**, **38**, **42**, and **43** indicates that detection of **5** should also be possible.

EXPERIMENTAL SECTION

General Methods. Solvents and reagents were purified as follows: diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone or purified using an Anhydrous Engineering solvent purification system using columns packed with A-2 Alumina; dichloromethane (CH_2Cl_2) was distilled from P_2O_5 or purified using an Anhydrous Engineering solvent purification system using columns packed with A-2 Alumina; CH_3CN was stirred over molecular sieves for 24 h, then distilled from P_2O_5; benzene, toluene, triethylamine, *i*-$PrEt_2N$, TMEDA, and DMPU were distilled from CaH_2; methanol was distilled over activated magnesium turnings, the purified reagents and solvents were used immediately or stored under nitrogen. Alkyl and aryllithiums were titrated using the procedure of Kofron[43] before use. Unless otherwise noted, all chemicals were used as obtained from commercial sources and all reactions were performed under nitrogen atmosphere in glassware dried in an oven (140 °C) or flame-dried and cooled under a stream of nitrogen. All reactions were stirred magnetically unless otherwise noted and liquid reagents were dispensed with all PP/PE plastic syringes or Hamilton Gastight microsyringes. Preparatory layer chromatography was performed using Whatman Partisil K6F silica gel 60 Å 200 or 1000 μm plates. Flash chromatography was performed with 230−400 mesh silica gel 60.

Isolation of Tetracyclic Alcohol 19. To a solution of indole **11** (0.69 mmol, 280 mg) in 1.8 mL THF and 0.9 mL DMPU (2:1) cooled to −78 °C was added a suspension of NaHMDS (0.759 mmol, 139.2 mg) in 0.75 mL THF cooled to −78 °C via cannula (1 drp/sec). After 5 min, aziridine **13** in 0.45 mL THF cooled to −78 °C was added dropwise into deprotonated indole via cannula (1 drp/sec). The cooling bath was removed and the reaction was warmed to rt while being concentrated to half the original volume under a stream of N_2. The reaction vessel was fitted with a reflux condenser and the solution was heated to 60 °C. After 3 d, the solution was cooled to rt and quenched with pH 3 phosphate buffer. The mixture was extracted 3 × Et_2O, the combined organics were washed with brine and dried over $MgSO_4$. Concentration under rotary evaporation provided 573 mg of a yellow oil. Purification by flash column chromatography (3:1 hexanes: ethyl acetate with 2% Et_3N) provided 124 mg product **14** pure by ^{1}H NMR and 36 mg (17%) desilylated **19**. The ^{1}H NMR spectrum of **14** matched that of Kim.[15] Spectroscopic data for **19**: 500 MHz ^{1}H NMR ($CDCl_3$, ppm) δ 7.67 (1H, s), 7.40−7.36 (6H, m), 7.21−7.13 (9H, m), 6.80 (1H, s), 6.68 (1H, s), 4.73 (2H, d, J = 6.1 Hz), 4.30 (2H, m), 4.23 (1H, dd, J = 14.0, 6.5 Hz), 4.09 (1H, dd, J = 14.0, 4.8 Hz), 3.98 (3H, s), 1.71 (1H, ddd, J = 18.9, 10.6, 6.8 Hz), 1.56 (1H, t, J = 6.1 Hz), 1.53−1.40 (6H, m), 1.36 (3H, t, J = 7.1 Hz), 1.34−1.24 (7H, m), 1.06−0.96 (6H, m), 0.87 (9H, t, J = 7.3 Hz).

***N*-Trityl Tetracyclic Ester 18 and *N*-Trityl Tetracyclic Aldehyde 7.** The procedure reported earlier[15] was modified as follows. A three-neck 100 mL round-bottom flask was fitted with a mechanical stirrer and thermocoupler. The flask was charged with a solution of **15**[15] (2.7 mmol, 2.68 g) 40 mL THF, and the solution was cooled to an internal temperature of −76 °C. A solution of MeLi in Et_2O (10.8 mmol, 1.51 M, 7.2 mL) was added slowly via syringe (max internal temp −74 °C). After 5 min of stirring, the solution was warmed to −65 °C and stirred for 20 min. A solution of PhSeCl in 14 mL THF (12.4 mmol, 2.37 g) was added slowly via cannula such that the reaction temperature did not exceed −58 °C, and the solution color became orange and then brown. The brown solution was removed from the cooling bath after 20 min and allowed to warm. When the temperature reached −18 °C, the flask was opened to air and stirred an additional 2 h. Opening of the reaction vessel to air was found to facilitate formation of **18** by oxidation and elimination of the selenium by product. The reaction was quenched with H_2O, extracted with $CHCl_3$, dried over Na_2SO_4 and allowed to sit overnight with exposure to air. ^{1}H NMR spectroscopy of the crude residue showed no noneliminated selenium tetracycle. The major product matched that of Kim.[15] The crude residue was filtered through a short plug of silica gel with 400 mL hexanes/2% Et_3N to remove excess PhSeCl, and then the product was eluted with 400 mL ethyl acetate. Limited exposure of this material to silica gel was necessary in order to retain a significant quantity of material. The material was immediately taken on to the next step without additional purification.

To the residue of **15** in 27 mL THF at 0 °C was added TBAF (1.0 M, 3.2 M, 3.2 mmol). The cooling bath was removed, and after 1 h 20 min of stirring at rt, the solution was cooled to 0 °C, and sat $NaHCO_3$ was added. The mixture was extracted 3 × Et_2O, the combined organics were washed with brine and dried over pulverized Na_2SO_4. Concentration under reduced pressure provided a brown oil consisting of tetracyclic alcohol product with minor impurities. The crude residue was filtered through a short silica gel plug with 400 mL 9:1 hexanes: ethyl acetate with 2% Et_3N and then 800 mL ethyl acetate. The residue obtained after solvent removal was immediately taken on to the next step without further purification. To a mixture of tetracyclic alcohol and 4 Å MS in 27 mL CH_2Cl_2 was added NMO (4.0 mmol, 469 mg) and TPAP (0.14 mmol, 49 mg). The black slurry was vigorously stirred for 1 h and then filtered through silica gel with ethyl acetate. Solvent removal provided an orange solid, which was dissolved in acetone and precipitated by adding hexanes. Washing of the recovered amorphous gray solid with hexanes provided 475 mg (33% over 3 steps, 69% average per step) of tetracycle **7**. The ^{1}H NMR spectrum matched that previously reported by Kim.[15]

(2*R*,3*R*)-3-Iodomethyl-2-tributylstannyl-*N*-triphenylmethylaziridine 20. To a solution of PPh_3 (0.56 mmol, 147 mg) in 2 mL toluene at 0 °C was added diisopropyl azodicarboxylate neat (0.56 mmol, 0.12 mL) via syringe, and the reaction mixture turned yellow.[18] After 5 min of stirring, aziridinol **12**[14] as a solution in toluene (0.37 mmol, 1.7 mL) was added to the PPh_3/DIAD mixture dropwise via cannula, and the mixture was stirred 5 min. Neat iodomethane (0.52 mmol, 0.03 mL) was added to the reaction mixture via syringe, and the mixture turned cloudy white. The reaction vessel was pulled out of the cooling bath, warmed to rt, fitted with a reflux condenser and heated to 70 °C. After 6 h, the reaction mixture was cooled to rt, and the solvent was removed under reduced pressure. The cloudy residue was purified immediately by flash column chromatography on silica gel (2 × 15 cm, 15:1 hexanes/ethyl acetate with 2% Et_3N, R_f = 0.82). Fractions 7−11 provided 257 mg (97%) of a clear colorless oil. Molecular ion (M − C_4H_9) calculated for $C_{30}H_{37}INSn$ = 658.0993, found (EI) m/z = 658.1004, error = 2 ppm; 500 MHz ^{1}H NMR ($CDCl_3$, ppm) δ 7.47 (6H, m), 7.27−7.24 (6H, m), 7.21−7.20 (3H, m), 3.57 (1H, dd, J = 10.0, 5.0 Hz), 3.00 (1H, dd, J = 9.5, 8.0 Hz), 1.62 (1H, ddd, J = 8.0, 6.8, 5.0 Hz), 1.5−1.4 (6H, m), 1.32 −1.24 (6H, m), 1.08−0.94 (7H, m), 0.86 (9H, t, J = 7.3 Hz); 100 MHz ^{13}C NMR

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($CDCl_3$, ppm) δ 144.1, 129.5, 127.3, 126.6, 76.0, 39.0, 29.8, 29.2, 27.3, 13.6, 10.8, 10.4.

Alkylation of 11; Preparation of Aziridinyl Indole 14. To a solution of **11**[15] (3.75 mmol, 1.52 g) in 9 mL THF and 4.6 mL DMPU (2:1) at −78 C, was added 1.0 M solution of NaHMDS (3.75 mmol, 3.75 mL) in THF via cannula dropwise (1 drop/sec). After 5 min of stirring at −78 °C, iodostannyl aziridine **20** in 2 mL THF was added dropwise via cannula. The cooling bath was removed and the reaction was warmed to rt while being concentrated by half under an N_2 stream. The reaction vessel was fitted with a reflux condenser and heated to 60 °C. After 3 h, the reaction was cooled in a 0 °C bath and quenched with satd NH_4Cl solution. The mixture was poured into water and extracted 3× with Et_2O, washed 2× with H_2O and then 1× with EtOAc, the combined organics were washed with brine and dried over $MgSO_4$. The organic solvent was removed under rotary evaporation to provide a dark brown oil as a mixture of product **14** and desilylated byproduct **19** (3:1 ratio) and minor unidentified byproducts. Purification by flash column chromatography (9:1 hex/EtOAc, 2% Et_3N) provided 860 mg (65%) of **14**. The NMR spectra of **14** matched those reported earlier.[15] An unknown contaminant multiplet was observed at 1.10 ppm.

Preparation of *N*-TBDPS Tetracyclic Aldehyde Ester 30. To a solution of **7** in 1.9 mL CH_2Cl_2 was added *i*-Pr_2EtN (0.02 mL, 0.14 mmol). The solution was cooled to −78 °C and TBDPSOTF[28] as a solution in CH_2Cl_2 (0.62 M, 0.16 mL, 0.096 mmol) was added dropwise via syringe (1 drop/5 s). After 30 min at −78 °C, the mixture was poured into pH 9.6 carbonate buffer, extracted 3 x with CH_2Cl_2 (10 mL), and the combined organic phases were washed with brine and dried over Na_2SO_4. Removal of the solvent by rotary evaporation provided a yellow oil, purified by flash chromatography on silica gel (2 × 15 cm, 2:1 hexanes/EtOAc with 2% Et_3N). Fractions 22−39 provided 37 mg (72%) of **30** as a pale yellow oil. Analytical TLC on silica gel 60 Å with 1:1 hexanes/EtOAc, R_f = 0.58. Molecular ion (M + Na) calculated for $C_{32}H_{34}N_2NaO_4Si$: 561.2186; found (electrospray) m/z = 561.2179, error = 1 ppm; IR (neat, cm^{-1}) 1721, 1690 C═O; 400 MHz ^1H NMR ($CDCl_3$, ppm) δ 9.98 (1H, s), 7.64−7.58 (4H, m), 7.48−7.32 (7H, m), 7.18 (1H, s), 4.29 (1H, dq, J = 12.0, 7.2 Hz), 4.27 (1H, d, J = 11.6), 4.08 (1H, m), 4.07 (1H, dd, J = 11.6, 3.6), 4.06 (3H, s), 3.62 (1H, d, J = 3.6 Hz), 3.38 (1H, dd, J = 3.6, 3.6 Hz), 1.15 (9H, s), 1.07 (3H, t, J = 7.2 Hz) 100 MHz NMR ^{13}C ($CDCl_3$, ppm) δ 191.8, 163.4, 154.7, 153.2, 135.85, 135.78, 134.7, 132.7, 131.9, 130.9, 129.8, 127.9, 127.7, 123.7, 107.6, 103.0, 101.3, 60.0, 56.0, 48.1, 40.6, 35.5, 27.6, 19.3, 14.0.

Pyrrole Hemiaminal 31. To a solution of pyrrole (0.10 mL, 1.44 mmol) in 3 mL THF at −78 °C was added *n*-BuLi as a solution in hexanes (0.87 mL, 1.5 M, 1.3 mmol) via syringe.[21,22] The resulting C_4H_4NLi was stirred at −78 °C for 20 min. To a solution of aldehyde **30** (0.020 mmol, 11 mg) in 0.40 mL THF at −78 °C was added C_4H_4NLi (1.3 M, 0.041 mmol) as a solution in 0.12 mL THF/hexanes. The resulting yellow solution was stirred at −78 °C for 15 min, and then $(CF_3)_2CHOH$ as a solution in Et_2O (0.05 mL, 0.95 M, 0.048 mmol) was added. The cooling bath was removed and while warming the solution was diluted with 2 mL cold Et_2O, and a small spatula tip of Celite was added to absorb insolubles. The organic phase was decanted from the insoluble material, and the solvent was removed under N_2. The residue was immediately purified by preparative TLC on silica gel 60 Å (20 cm ×20 cm x 1000 μm) pretreated with Et_3N vapors for ≥2 h to give **31** after extraction and solvent removal as a 1:1 mixture of diastereomers containing a trace of aldehyde **30** (6%) and residual Et_3N. Attempted removal of Et_3N under vacuum increased the conversion to aldehyde **30** due to loss of pyrrole hemiaminal. Characterization of **31**: 2:1 hexanes/EtOAc, R_f = 0.28. IR (neat, cm^{-1}) 3411, OH; 1696, C═O. 500 MHz ^1H NMR ($CDCl_3$, ppm) δ 7.64−7.58 (4 H, m, Si-*Ph*$_2$), 7.48−7.32 (6H, m, Si-*Ph*$_2$), 6.88−6.82 (2.7H, m, pyrrole + C(7)*H*), 6.68−6.60 (1.9H, m, C(OH)*H* + C(5)*H*), 6.21 (1.7H, m, pyrrole), 4.31−4.22 (1.4H, m, OEt), 4.17 (1.0H, d, J = 11.2 Hz, C(3)H_aH_b), 4.10−4.02 (1.2H, m, OEt), 3.95−3.93 (1H, m, C(3)H_aH_b), 3.94−3.93(3H, two partly overlapping singlets, OCH_3), 3.56 (0.95H, d, J = 3.6 Hz, C(1)*H*), 3.31 (1H, dd, J = 3.6 Hz, 3.2 Hz, C(2)*H*), 1.14−1.13 (8.8H, Si-*t*-*Bu*, two partly overlapping singlets), 1.04 (8.9H, m, ethyl ester and residual Et_3N). The presence of Et_3N was confirmed by a quartet at δ 2.56. Two diastereomers were expected and confirmed by the presence of two OCH_3 singlets (δ = 3.94 and 3.93 ppm) and two *t*-Bu singlets at δ = 1.14 and 1.13 ppm. All other signals of the diastereomers could not be distinguished by ^1H NMR spectroscopy; relative integral values are based on the clean doublet at 4.17 ppm = 1.0H (both diastereomers).

Conversion of 31 to Tetracyclic Carbamate Aldehyde 36. To a solution of pyrrole hemiaminal **31** (48 mg, 0.08 mmol) in 1.3 mL Et_2O at 0 °C was added LAH in a solution of THF (0.42 mL, 1.0 M, 0.40 mmol, 5 mol equiv) dropwise via syringe. The cloudy nonhomogeneous mixture was stirred vigorously at 0 °C. After 50 min, 0.5 mL EtOAc was added slowly at 0 °C, the mixture was allowed to warm to rt, and was stirred for an additional 1.5 h under air. The crude mixture was then filtered through Celite with ethyl acetate (4 × 10 mL), and the combined organic washes were concentrated under reduced pressure to provide 25 mg of alcohol **33** and **32** (14:1 **33**:**32**) as a clear green oil (63% mass recovery). In preliminary experiments the stirring time at rt prior to Celite treatment was shorter, and resulted in ratios of **33**:**32** in the range of 1−3:1. For characterization purposes, the mixture of **33** and **32** and byproducts was purified on an analytical TLC plate on silica gel 60 Å (20 cm × 20 cm × 100 μm) pretreated with Et_3N vapors for ≥1 h with 1:1 hexanes: ethyl acetate but most of **32** decomposed during separation and an enriched sample could not be obtained. Selected data for **32** (observed in the mixture prior to separation): 400 MHz ^1H NMR ($CDCl_3$, ppm) δ 4.93 (1H, **A**BX dd, J = 12.4, 5.3 Hz), 4.73 (1H, ABX dd, J = 12.4, 8.2 Hz), 3.28 (1H, dd, J = 3.4, 3.4 Hz), 3.22 (1H, d, J = 4.0 Hz), 2.99 (1H, AB**X** dd, J = 8.2, 5.3 Hz). Characterization data for **33** (purified; R_f = 0.44): IR (neat, cm^{-1}) 3539 OH, 1681 C═O; 400 MHz ^1H NMR ($CDCl_3$, ppm) δ 9.95 (1H, s), 7.62−7.58 (4H, m), 7.48−7.36 (6H, m), 7.32 (1H, s), 7.08 (1H, s), 4.95 (1H, **A**BX dd, J= 12.8, 5.6 Hz), 4.76 (1H, ABX dd, J = 12.8, 8.0 Hz), 4.26 (1H, d, J = 11.2 Hz), 4.06 (3H, s), 4.03 (1H, ABX dd, J = 11.2, 3.2 Hz), 3.4 (1H, AB**X** dd, J = 3.6, 3.2 Hz), 3.29 (1H, d, J = 3.6 Hz), 2.88 (1H, AB**X** dd, J = 8.0, 5.6 Hz), 1.15 (9H, s).

Because purification resulted in considerable material loss due to decomposition, the crude 14:1 mixture of alcohols **32** and **33** (25 mg, 0.05 mmol) was used directly in the following step. Thus, **32**+**33** in 1 mL CH_2Cl_2 was cooled to −78 °C, charged with Et_3N (0.72 M, 0.06 mmol) in 0.08 mL of CH_2Cl_2 and Fmoc-NCO[31] (0.47 M, 0.10 mmol) in 0.21 mL of CH_2Cl_2 was added via syringe. After 20 min of stirring at −78 °C, the yellow green solution was warmed to rt and stirred for 1 h (the mixture turned dark yellow upon warming). The CH_2Cl_2 was removed under N_2, and the crude residue was dissolved in 1 mL THF and cooled to 0 °C. Next, TBAF on Al_2O_3[32,33] (436 mg, 0.25 mmol) was added in one portion and the mixture was stirred and warmed to rt in the cooling bath. After 1.5 h, the crude reaction mixture was filtered through Celite with EtOAc and the solvent was removed under reduced pressure to provide 22 mg of residue. The residue was purified twice by preparative TLC on silica gel 60 Å (20 cm ×20 cm x 250 μm) pretreated with Et_3N vapors for ≥30 min, providing **36** (20 mg, 74%) as a white amorphous powder; 1:1 hexanes/EtOAc; R_f = 0.28; Molecular ion calculated for (M − CO_2NH_2) $C_{30}H_{31}N_2O_2Si$: 479.2149; found (electrospray) m/z = 479.2168, error = 4 ppm; IR (neat, cm^{-1}) 3473, 3348, $CONH_2$; 400 MHz ^1H NMR ($CDCl_3$, ppm) δ 9.94 (1H, s), 7.63−7.61 (4H, m), 7.43−7.38 (6H, m), 7.31 (1H, s), 7.05 (1H, s), 5.47 (2H, ABq, J = 12.2 Hz), 4.41(2H, br s), 4.23 (1H, d, J = 11.2 Hz), 4.02 (1H, ABX dd, J = 11.2, 3.2 Hz), 3.97 (3H, s), 3.41 (1H, d, 4.0 Hz), 3.36 (1H, ABX dd, J = 4.0, 3.2 Hz), 1.15 (9H, s); 100 MHz ^{13}C NMR ($CDCl_3$, ppm) δ 192.0, 156.9, 154.6, 146.5, 135.9, 135.8, 132.1, 132.0, 130.0, 127.9, 127.8, 125.1, 104.5, 98.2, 59.6, 55.5, 47.5, 41.4, 33.2, 27.5, 19.3.

ttempted Conversion Tetracyclic Alcohol 33 to 36 using Trichloroacetyl Isocyanate. To a solution of **33** (0.0046 mmol, 2.28 mg) in 0.30 mL CH_2Cl_2 at −78 °C was added trichloroacetyl isocyanate as a solution in CH_2Cl_2 (0.28 M, 0.0051 mmol, 18 μL) via microsyringe. The dark yellow solution was stirred at −78 °C for 1 h, and then allowed to warm to rt. After 1 h, pH 9.6 carbonate buffer was added and the mixture was stirred for 5 min, extracted 3× with

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CH_2Cl_2, washed 1× with brine, and dried over pulverized Na_2SO_4. Solvent removal provided a yellow film. Analysis of the residue by ^1H NMR spectroscopy ($CDCl_3$, ppm) showed an AB doublets at δ = 5.66 (J = 12.0 Hz) and 5.56 (J = 11.5 Hz) in a complex spectrum. The crude residue was dissolved in 0.30 mL MeOH and 0.30 mL K_2CO_3 (5% w/v) in MeOH[29] and stirred vigorously for 2.5 h. The mixture was extracted with ethyl acetate and solvent removal under reduced pressure provided an orange film. Analysis by ^1H NMR spectroscopy showed loss of the characteristic AB doublets in a complex spectrum lacking diagnostic signals.

(a) **Attempted Desilylation of Tetracycle 30 using Weak Silaphiles.** Treatment with D_2O in CD_2Cl_2: a residue of **30** (2.3 mg) was dissolved in CD_2Cl_2 saturated with D_2O, and the sample was monitored by ^1H NMR spectroscopy. After 3 h no reaction was observed, so the NMR tube was heated to 35 °C (oil bath); no deprotected **7** was detected after 2 h of heating.

(b) Treatment with 2,2,2-trifluoroethyl alcohol in $CDCl_3$: a solution of **30** (2.3 mg) in $CDCl_3$ was treated with excess TFE (ca. 30 μL), and the sample was monitored by ^1H NMR spectroscopy. No change was seen after 3 h. The sample was gently heated with an oil bath to 35 °C; no deprotected **7** was observed after 20 h.

(c) CD_3OD: **30** (2.3 mg) was dissolved in CD_3OD and monitored by ^1H NMR spectroscopy. New peaks consistent with formation of **7** were apparent between δ = 4.4 and 3.4 ppm after 1.5 h, and the relative intensities continued to increase at 3 and 5 h. Also, two new signals appeared upfield: a triplet at δ = 1.43 ppm corresponding to the CH_3 signal of the ethyl ester, and a singlet at δ = **1.01** ppm corresponding to a new TBDPS species ($TBDPSOCD_3$). After 20 h, no starting material remained by ^1H NMR, and the spectrum showed only the known NH aziridine **7**[15] with chemical shifts as follows: 400 MHz ^1H NMR (CD_3OD, ppm) δ 9.94 (1H, s), 7.72−7.66 (4H, m), 7.56 (1H, s), 7.48−7.38 (6H, m), 7.21 (1H, s), 4.37 (2H, q, J = 7.2 Hz), 4.28 (1H, br s), 4.04−3.96 (4H, m), 3.75 (1H, br s), 1.43 (3H, t, J = 7.2).

(d) Cs_2CO_3/CD_3OD: A residue of TBDPS aziridinomitosene **30** was dissolved in a solution of Cs_2CO_3 (5 mg) in 0.5 mL CD_3OD.[44] The sample was monitored by ^1H NMR spectroscopy over 5 h. No change was observed after 5 min, but after 15 min, a triplet, corresponding to the ethyl ester group of **7** was observed at δ = 1.43 ppm and a singlet, corresponding to a new TBDPS species, was observed at δ = 1.01 ppm. After 1 and 4 h, the signal intensity of both the triplet and singlet had increased. At 5 h, the relative amount of **7** was determined to be 12% by integration of the unobscured triplet at 1.43 ppm.

(a) **Attempted Desilylation of Tetracycle 36 using Oxygen Silaphiles.** CD_3OD: Tetracycle **36** was dissolved in CD_3OD and monitored by ^1H NMR spectroscopy over a 4 h period. After 15 min, the aziridine C(1) and C(2) protons at δ = 3.53 and 3.49 ppm, as well as the C(10) methylene protons at δ = 5.41 and 5.34 ppm began to decrease in intensity. The singlet at 1.09 ppm corresponding to the *tert*-butyl of the TBPDS group was less intense, while a singlet at 1.01 ppm began to appear. At 45 min, the intensity of the C(1), C(2) and C(10) peaks decreased further, but no new aziridine methine peaks appeared. By 2 h, the C(1) and C(2) peaks were no longer visible, and after 4 h, the singlet at 1.09 ppm had disappeared. No signals corresponding to **9** were observed; however, the presence of a *tert*-butyl signal at 1.01 ppm is consistent with desilylation to form $TBDPSOCD_3$. After 2 days, the sample showed signals near 4.6 ppm and 3.8 ppm, consistent with aziridine ring-opening.

(b) Cs_2CO_3/CD_3OD; detection of **37** and **38**: Tetracycle **36** (ca. 5 mg, crude residue contaminated with FMOC-NH_2 and dibenzofulvene) was dissolved in a mixture of Cs_2CO_3 (5 mg) and 0.5 mL CD_3OD and the sample was monitored by ^1H NMR spectroscopy every 15 min for 10 h. After 1 h, four new methine signals began to appear (δ 3.5−3.4 ppm and 2.6−2.4 ppm) and a new ABX pattern was observed (δ 4.7−4.9 ppm). The intensity of these signals continued to increase during a 10 h period, and at 17 h, all four methine signals were still present. However, after 6 d, the methine protons near δ = 3.5−3.4 ppm had disappeared, while the signals near δ = 2.6−2.4 ppm were still present. After evaporation, the material was applied to an analytical TLC plate, silica gel 60 Å (20 cm × 20 cm × 250 μm) pretreated with Et_3N fumes (≥30 min) and immediately developed sufficient to advance the mobile zone beyond poorly defined polar zones streaking up from the origin. The silica gel containing the mobile zone was collected rapidly, and immediately extracted to give material containing the highly sensitive **37**, FMOC-NH_2, dibenzofulvene, and other unknown contaminants. Partial data for **37**: Molecular ion (M + H) calcd for $C_{31}H_{32}D_3N_2O_3Si$ = 514.3, found (electrospray, nominal mass) m/z = 514.3; 500 MHz ^1H NMR ($CDCl_3$, ppm) δ 9.92 (1H, s), 7.45−7.30 (m), 6.89 (1H, s), 6.78 (1H, s), 6.29 (1H, d J = 1.0 Hz), 5.55 (1H, d J = 1.0 Hz), 4.01 (3H, s), 3.55 (1H, d, J = 12.0 Hz), 3.34 (1H, d, J = 12.0, 1.4 Hz), 2.66 (1H, d, J = 3.1 Hz), 2.43 (1H, dd, J = 3.1, 1.4 Hz), 0.84 (9H, s); singlet at δ = 6.08 ppm due to dibenzofulvene; doublet at δ = 4.42 ppm due to FMOC-NH_2. Partial data for **38** (based on signals in the mixture prior to chromatography; not isolable): 500 MHz ^1H NMR ($CDOD_3$, ppm) δ 4.79 (2H, ABq, J = 11.3 Hz), 3.50 (1H, m), 3.40 (1H, d, J = 3.5 Hz).

Heterolysis of Tetracycle 40 with CD_3OD/Cs_2CO_3; Detection of 41 and 42. Deuterated methanol (0.50 mL) was stirred with Cs_2CO_3 (10 mg, 0.03 mmol) and the mixture was allowed to settle. The supernatant liquid was decanted away from insoluble material, and then added to **40** (<15 mg).[15] The solution was monitored repeatedly at rt by ^1H NMR spectroscopy. After 1.5 h, the ratio of **40**:**41**:**42** was 75:11:14 according to integration, and 12:42:46 after 26 h. After 67 h, 3:40:57 of **40**:**41**:**42**, the solvent was removed under N_2 flow and the residue was purified on an analytical TLC plate on silica gel 60 Å (20 cm × 20 cm × 250 μm) pretreated with Et_3N fumes (≥30 min) with 2:1 pentane/ethyl acetate providing small amounts of **41** and **42** as yellow oils. The less polar fraction was identified as **41**: molecular ion (M + Na) calcd for $C_{34}H_{27}D_3N_2NaO_3$ = 540.2342, found (electrospray with formic acid, for optimum ionization) m/z = 540.2360, error = 3 ppm; 500 MHz ($CDCl_3$, ppm) δ 9.97 (1H, s), 7.26−7.20 (6H, m), 7.18−7.12 (9H, m), 6.96 (1H, d, J = 0.5 Hz), 6.93 (1H, s), 6.29 (1H, d, J = 1.0 Hz), 5.47 (1H, d, J = 0.5 Hz), 4.11 (3H, s), 3.80 (1H, d, J = 12.5), 3.40 (1H, dd, J = 12.5, 1.5 Hz), 2.28 (1H, d, J = 4.5 Hz), 2.13 (1H, dd, J = 4.5, 1.5) 125 MHz ^{13}C ($CDCl_3$, ppm) δ 192.1, 157.1, 156.8, 144.1, 140.2, 138.8, 129.3, 127.4, 126.6, 120.9, 114.4, 106.1, 104.0, 103.9, 73.8, 55.6, 51.5, 45.3, 37.0, the OCD_3 carbon was not detected. The polar fraction was identified as **42**: molecular ion (M + Na) calcd for $C_{34}H_{27}D_3N_2O_3$ = 540.2342, found (electrospray with formic acid) m/z = 540.2341, error = 0.2 ppm; 500 MHz ^1H NMR ($CDCl_3$, ppm) δ 9.97 (1H, s), 7.49 (6H, d, J = 7.5 Hz), 7.38 (3H, d, J = 14.0 Hz), 7.30 (6H, t, J = 7.5 Hz), 7.25 (1H, m), 7.07

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(1H, s), 4.81 (2H, ABq, J = 11.5 Hz), 4.44 (1H, d, J = 11.0 Hz), 4.10 (1H, dd, J = 11.0, 3.5 Hz), 3.04 (1H, d, J = 5.0 Hz), 3.01 (1H, dd, 5.0, 3.5 Hz); 125 MHz ^{13}C NMR ($CDCl_3$, ppm) δ 192.1, 154.8, 145.3, 144.1, 133.8, 131.8, 129.3, 128.3, 127.8, 127.1, 125.7, 109.0, 107.1, 98.1, 74.5, 66.2, 55.5, 47.4, 42.5, 35.1.

(a) **Desilylation Attempts with 44 by Treatment with Silaphiles.** TBAF: To a solution of aziridine **44** (0.24 mmol, 108 mg) in 2.4 mL DMF at rt was added TBAF as a solution in THF (1.0 M, 0.24 mL). After 3 h of stirring at rt, saturated $NaHCO_3$ (1.0 mL) was added. The mixture was extracted 3× with Et_2O, the combined organics were washed with brine, and then dried over $MgSO_4$. Removal of the solvent by rotary evaporation provided an oil, and flash chromatography on silica gel (2 × 15 cm silica gel, 1:1 hexanes/Et_2O, R_f = 0.18) provided 28 mg (56%) of desilylated aziridine **45**.[36]

(b) TBAT: Aziridine **44** was dissolved in ca. 1 mL DMF-d_7, and a spatula tip of TBAT was added to the solution. Only **44** and TBAT were observed by ^{1}H NMR spectroscopy after 3 d. To a sample of **44** (0.58 mmol, 25.9 mg) in 1 mL DMF-d_7 was added TFE (0.58 mmol, 4.2 μL) and TBAT (0.58 mmol, 32 mg). The sample was monitored by ^{1}H NMR spectroscopy. At 1 h, peaks corresponding to **45** had appeared; at 2 h, 27% conversion was determined by integration. After 24 h, 75% of **45** was present.

(c) CsF and TFE: An oven-dried NMR tube was charged with CsF (0.10 mmol, 15 mg) and capped with a rubber septum under nitrogen. A solution of aziridine **44** in DMF-d_7 (0.06 mmol, 27.5 mg, 0.5 mL) was then added via syringe. Neat trifluoroethanol (TFE, 0.06 mmol, 4.3 μL) was then added, the NMR tube was shaken for 30 s, and the insoluble material was allowed to settle. The sample was monitored by ^{1}H NMR spectroscopy without spinning. After 10 min, **45** began to appear, and after 2 h, 48% of **45** was present. At 20 h, 93% conversion to **45** was observed.

(d) CsF + TFE/CH_3CN: To a nonhomogenous solution of **44** (0.26 mmol, 114.1 mg) in 1.8 mL CH_3CN was added CsF (0.30 mmol, 47 mg) as a solution in CH_3CN (0.8 mL) and TFE (0.10 mL, 1.39 mmol). The resulting white nonhomogenous mixture was stirred vigorously. The solution cleared after 3 d of stirring, and a saturated solution of $NaHCO_3$ was added. The mixture was extracted 3 x with Et_2O, the combined organics were washed with brine, and dried over $MgSO_4$. Removal of the solvent under rotary evaporation provided a clear yellow oil. The residue was purified by preparatory plate TLC on silica gel 60 Å (20 cm × 20 cm × 1000 μm) pretreated with Et_3N fumes (≥30 min) with 9:1 hexane/ethyl acetate. Elution of the bands with ethyl acetate provided 13 mg (11%) of recovered **44** and 22 mg (41%) of **45**.

(e) CsF/TFE: To a solution of aziridine **44** (0.33 mmol, 147 mg) in 1.3 mL TFE was added CsF as a solution in TFE (0.17 M, 2.0 mL, 0.33 mmol). Aziridine **44** was insoluble in TFE, so the reaction vessel was placed in a sonicator to break up the insoluble material. Then the suspension was stirred vigorously at rt. After 2 d of stirring, 2 mL of satd $NaHCO_3$ was added, and the mixture was extracted 3× with Et_2O, washed 1× with brine, and dried over $MgSO_4$. Solvent removal under rotary evaporation provided an oily residue. The crude residue was purified by flash chromatography with silica gel (2 × 15 cm, 3:1 hexanes/ethyl acetate, with 2% Et_3N in the eluent). Fractions 25−32 provided 42 mg (61%) of **45**.

Attempted Desilylation of 36 with CsF in TFE; Isolation of 46. To a solution of **36** (0.0019 mmol, 1 mg) in 0.05 mL THF was added TFE (5.6 mmol, 0.40 mL). The cloudy solution cleared and turned bright yellow. A solution of CsF in TFE (0.11 M, 0.0022 mmol, 20 μL) was then added via microsyringe. After no apparent reaction by TLC, another equiv of CsF was added at 2 and 5 h, and 2 equiv of CsF was added at 6.5 h for a total of 5 equiv of CsF. At 24 h, the solvent was removed under a flow of N_2, and the orange residue was purified by preparatory plate TLC on silica gel 60 Å (20 cm × 20 cm × 250 μm) pretreated with Et_3N fumes (≥30 min) with 100% ethyl acetate to provide one band consisting of a mixture of two products in approximately a 1:1 ratio contaminated with unknown by products. Repeated purification by preparatory plate TLC on silica gel 60 Å (20 cm × 20 cm × 250 μm) pretreated with Et_3N fumes (≥30 min) with 5:1 hexanes/ethyl acetate provided a zone at R_f = 0.27 consisting of the major diastereomer **46b** containing unknown contaminants; molecular ion (M + Na) calculated for $C_{34}H_{36}F_6N_2NaO_4Si$ = 701.2, found (electrospray, nominal mass) m/z = 701.3; 500 MHz partial ^{1}H NMR ($CDCl_3$, ppm) δ 9.91 (1H, s), 7.70 (2H, dd, J = 8.1, 1.4 Hz), 7.65 (2H, dd, J = 8.1, 1.4 Hz), 7.46−7.37 (7H, m), 7.08 (1H, s), 5.09 (1H, d, J = 11.8 Hz), 4.99 (1H, d, J = 11.8 Hz), 4.74 (1H, s), 4.34 (1H, dd, J = 10.5, 5.5 Hz), 4.06−4.00 (1H, m), 4.00 (3H, s), 3.93 (2H, q, $J_{H,F}$ = 8.8 Hz), 3.89 (1H, dd, J = 10.5, 2.0 Hz), 3.56−3.44 (2H, dq, J = 12.3 Hz, $J_{H,F}$ = 8.6), 1.01 (9H, s); 376 MHz ^{19}F ($CDCl_3$, ppm) δ −74.0 (t, $J_{F,H}$ = 8.8 Hz), −74.4 (t, $J_{F,H}$ = 8.6 Hz). Impurity signals were present δ 4.12 (EtOAc), 2.25−2.20, 2.04 (EtOAc), 2.04−1.98, 1.67−1.60, 1.54 (H_2O), 1.40−1.20 (EtOAc), 1.01 (s), 0.91−0.78 ppm. The minor diastereomer **46a** (tentative assignment), R_f = 0.42 could not be obtained free of **46a** and unknown contaminants; molecular ion (M + Na) calculated for $C_{34}H_{36}F_6N_2NaO_4Si$ = 701.2, found (electrospray, nominal mass) m/z = 701.3; 500 MHz partial ^{1}H NMR ($CDCl_3$, ppm) δ 9.94 (1H, s), 5.11 (1H, d, J = 12.0 Hz), 4.85 (1H, d, J = 12.0 Hz), 4.56 (1H, d, J = 4.5 Hz), 4.09 (1H, m), 3.98−3.88 (4H, m), 3.84 (1H, m); 376 MHz ^{19}F NMR (C_6D_6, ppm) δ −73.7 (dd, J = 9.0, 8.6 Hz), −73.9 (dd, J = 9.7, 8.3 Hz).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of new compounds and experimental details for **24-28** and **44** are reported in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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