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SYNTHESIS AND BIOLOGICAL ACTIVITY OF C-4 AND C-15 ARYL AZIDE DERIVATIVES OF ANGUIDINE

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Abstract. Potential trichothecene photoaffinity reagents were prepared by coupling either the C-4 or C-15 alcohols derived from anguidine with (3-azido-5-methoxyphenoxy)acetic acid, 4-(3-azido-5-methoxyphenoxy)butyric acid, or N-(3-azido-5-methoxyphenyl) N'-(carboxymethyl) urea. The C-15 anguidine deriviatives of (3-azido-5-methoxyphenoxy)acetic acid and (3-azido-4-iodo-5-methoxyphenoxy)acetic acid possessed protein synthesis inhibition activity comparable to that of anguidine itself in Chinese hamster ovary and African Green Monkey kidney cell lines.

A substantial body of evidence suggests that the trichothecenes[1] are protein synthesis inhibitors acting at the ribosomal level,[2] but several lines of evidence suggest a multiplicity of binding sites for the trichothecenes within the ribosomal complex. Efforts to define the locus of this interaction include studies[3] of resistant strains of certain yeast, which possess a eukaryotic ribosomal system characteristic of mammalian cells or studies[4] of the ribosomal subunits of the trichothecene-producing fungi themselves, which possess a less complicated prokaryote ribosomal system. In order to address the question of the ribosomal binding site or sites for the trichothecenes, we have undertaken the preparation of suitable photoaffinity reagents[5] of the trichothecenes. Such probes must possess a suitable "reporter group" such as an aryl azide, a radioisotope of high specific activity, and a link to the trichothecene which does not impair the biological activity exhibited by the parent system. The confluence of these factors presents an interesting synthetic challenge, and we wish to report a solution to these problems in which the modification of the C-15 position of anguidine (1) with an aryl azide capable of iodination produced a biologically active derivative.

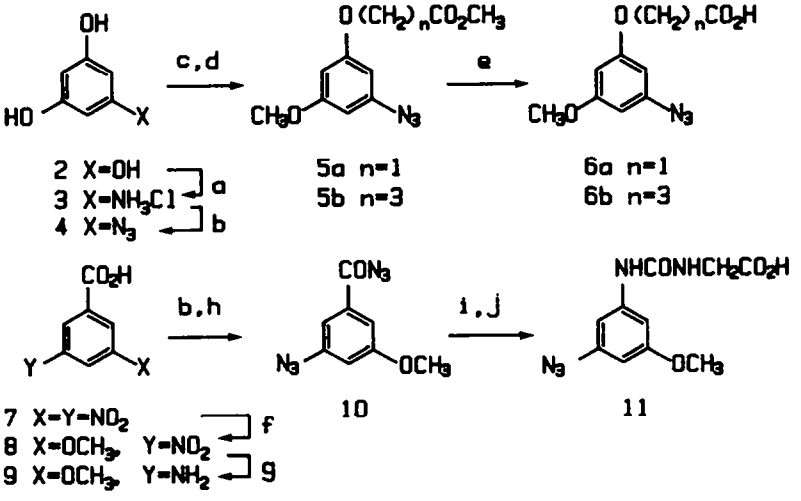
Although many photoaffinity reagents possess aryl azides bearing electron withdrawing groups, we required an aryl azide which was activated toward electrophilic aromatic substitution in order to introduce the radioiodine label as the last step in the synthesis without disrupting the chemically sensitive trichothecene. Since the number of copies of the trichothecene receptor as well as the efficiency of the photochemical cross-linking experiment was uncertain, we also needed to incorporate a radiolabel of high specific activity. Since an ^{125}I radiolabel would fulfill this requirement

and would possess a convenient half-life, we developed a mild iodination procedure[6,7] that was compatible with the trichothecene functionality. The deactivating nature of the azide group[8] on the electrophilic aromatic substitution reaction needed to introduce the radiolabel was surmounted by the presence of either one activating hydroxyl group or two meta-oriented alkoxy groups. This latter requirement led, in the specific case of the trichothecenes under discussion here, to the preparation of an aromatic "reporter" group which possessed: (1) the azide group; (2) an alkoxy group which serves to facilitate the iodination; and (3) a second alkoxy group which served both to activate the aromatic ring and to link the "reporter" group to the trichothecene.

The crucial issue that we needed to address involved the selection of those regions of the trichothecene structure which could be modified without dramatically altering the biological activity of these toxins. A report[9] by Kaneko indicating that various trichothecenes in which the C-4 acetate group was replaced by a C-4 chloroacetate group retained biological activity suggested the introduction of other α-heteroatom substituents in the C-4 or C-15 acetate groups as a means of introducing the desired "reporter" group. In particular, we replaced either the C-4 or the C-15 acetate groups with a glycolate or glycinate derivative, which was used to establish a link between the trichothecene and the "reporter" group.

As shown in Scheme 1, the preparation of the glycolic acid derivatives 6 involved a straightforward reaction sequence: [1] conversion of phloroglucinol (2) to 5-azidoresorcinol (4); [2] monoalkylation of 4 with methyl bromoacetate or methyl 4-bromobutyrate; [3] methylation; and [4] saponification. Preparation of a glycine derivative 11 involved an equally straightforward sequence: [1] nucleophilic aromatic substitution of 3,5-dinitrobenzoic acid (7) by lithium methoxide[10] to afford 8; [2] conversion of the nitro group to an azide; [3] Curtius rearrangement; and [4] the addition of glycine to the intermediate isocyanate. Model studies using 1,3-dicyclohexylcarbodiimide to effect the coupling of the carboxylic acids 6 or 11 to exo-norborneol were employed in order to optimize conditions for the trichothecene esterifications.

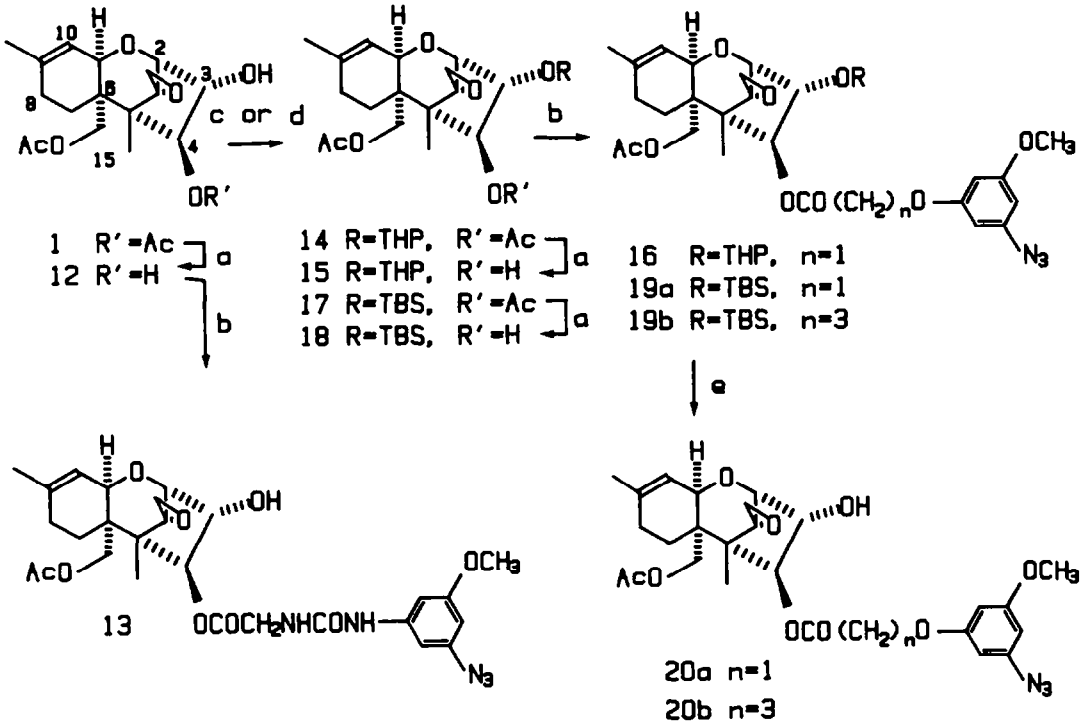
Scheme 1.



a, NH_3, HCl; b, $NaNO_2$, HCl followed by NaN_3; c, NaH, $Br(CH_2)_nCO_2Me$; d, Me_2SO_4, K_2CO_3; e, KOH, aqueous EtOH; f, LiOMe, HMPA; g, H_2, Pd-C; h, $ClCO_2CH_3$ followed by NaN_3; i, heat; j, glycine, NaOH

Using the procedure of Pathre,[11] we selectively saponified the C-4 acetate of anguidine (1) to obtain the diol (12) as shown in Scheme 2. Acylation[12] of 12 with a carboxylic acid such as 11 led to a mixture of C-3 and C-4 acylated material from which the ester 13 was isolated. To avoid such mixtures, protection of the C-3 hydroxyl group in anguidine (1) with dihydropyran[9c,13] furnished the THP derivative 14 which was selectively saponified[11] to give 15 and acylated to give 16, but the deprotection of 16 failed under a variety of conditions.[13] Protection of the C-3 hydroxyl group in 1 as the tert-butyldimethylsilyl (TBS) ether[14] 17 led to a derivative which was selectively saponified[11] at C-4 to give 18, coupled to a carboxylic acid such as 6 to give 19, and deprotected to give the ester 20.

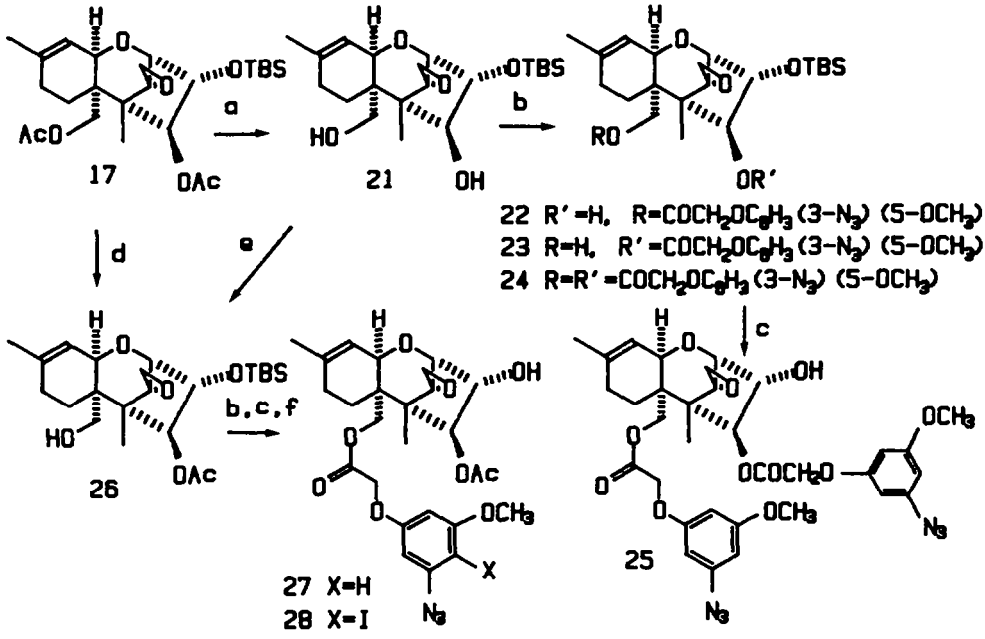
Scheme 2.



a, NaOH, NaOAc, aqueous MeOH; b, RCOOH 6 or 11, DCC, 4-(dimethylamino)pyridine or 4-(pyrrolidino)pyridine; c, DHP, PPTS; d, TBSCl, imidazole, DMF; e, nBu_4NF, THF

During the saponification of 17, we obtained a small amount of the diol 21 shown in Scheme 3. Coupling of 6 to the diol 21 led, not unexpectedly, to a mixture of the C-15 adduct 22, the C-4 adduct 23, and the C-4,15 bisadduct 24, from which only the bisadduct 24 was obtained in a pure state. Deprotection of the TBS group at C-3 in 24 furnished the unusual "double" ester 25. In order to prepare the C-15 ester 27 shown in Scheme 3, we examined the selective acetylation[15] of the C-4 hydroxyl group in diol 21 in order to secure the acetate 26; however, a more efficient route to the acetate 26 involved the selective saponification[16] of the C-15 acetate group in 17. The esterification of 26 with the acid 6 and deprotection of the TBS group led to the ester 27.

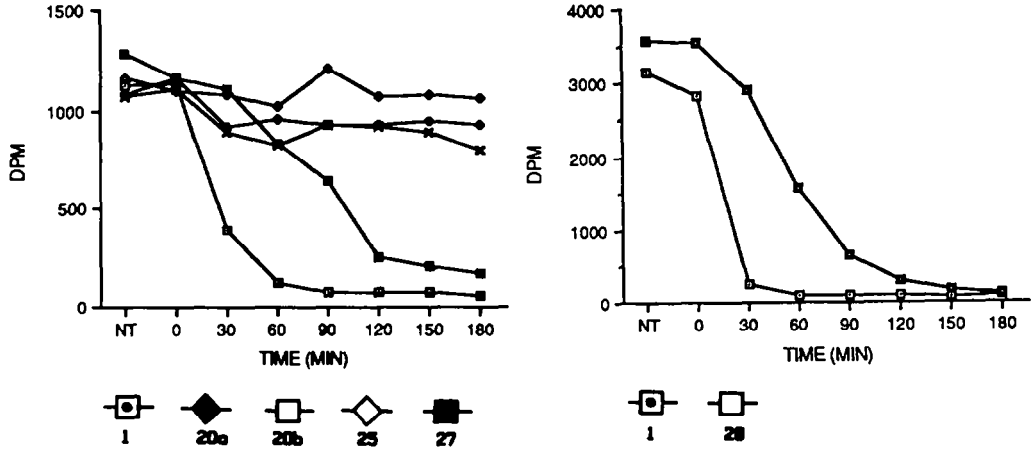
Scheme 3.



a, NaOAc, NaOH, aqueous MeOH; b, RCOOH 6, DCC, 4-pyrrolidinopyridine; c, nBu_4NF; d, LiOH, CH_3OH, DME; e, N-acetylimidazole, DBU; f, NaI, t-BuOCl.

As a first step in the evaluation of these derivatives as potential photoprobes, compounds 13, 20a, 20b, 25, and 27 were evaluated in a protein synthesis inhibition assay using either Chinese hamster ovary (CHO) or African green monkey kidney (VERO) cell lines. Modification of anguidine (1) at the C-4 position decreased biological activity significantly as shown in Figure 1. Modification of the C-15 position, however, produced a promising derivative 27 which was comparable in activity to 1. The C-15 monoadduct 27 was more active than the C-4,15 bisadduct 25. The iodinated derivative 28 of the C-15 photoprobe retained the same level of activity as 1, augering well for the utilization of the radioiodinated analog in photolabeling experiments.

Figure 1. Protein Synthesis Inhibition by Anguidine (1) and C-4 and C-15 Aryl Azide Derivatives in VERO Cells.



Acknowledgement

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Experimental Section

CAUTION: Trichothecenes are extremely toxic and all intermediates should be handled in a fume hood using plastic gloves.

Infrared spectra were determined on a Beckman Microlab 600 or Perkin Elmer 357 instrument. The abbreviation TF denotes thin film. NMR spectra were determined on a Varian EM390 or Varian XL-200 spectrometer. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. Organic extracts were routinely washed with brine and dried over anhydrous magnesium sulfate. Preparative layer and column chromatography were performed using Macherey Nagel silica gel F254 or 60, respectively, and is referred to as "silica gel chromatography." Abbreviations: DCC = 1,3-dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine, PPTS = pyridinium p-toluenesulfonate, DME = 1,2-dimethoxyethane, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

Cultured cell lines used in biological experiments consisted of Chinese hamster ovary (CHO) and African green monkey kidney (VERO) cells (American Type Culture Collection, Rockville, MD). Cells were maintained in Earles minimal essential medium (EMEM) (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum containing 100 units/mL of penicillin, 100 µg/mL of streptomycin, 50 µg/mL gentamycin, and 2.5 µg/mL of fungizone. Cultures were incubated in a 37°C, 5% CO_2 incubator. CHO and VERO cells were seeded into 24-well tissue culture dishes (Costar, Cambridge, MA) at a density of 1 x 10^5 cells/well and incubated in a 37°C warm box overnight. Medium H-199 (GIBCO, Grand Island, NY) was supplemented with 10% fetal calf serum, 25 mM HEPES buffer, 50 µg/mL of gentamycin, and 2.5 µg/mL of fungizone. The following morning, the medium was removed; fresh medium was added; and the cells were allowed to equilibrate for 1 h. At the appropriate time, the media was removed, and 1 mL of fresh media was added which contained either no toxin (controls), or the natural toxin, anguidine (1), at a concentration of 0.01µg/mL. Toxin was added in a reverse time sequence (i.e, longest exposure first) so that all wells could be processed simultaneously. At the appropriate time, the toxin was removed, and the tritiated [^{3}H]-leucine (144 Ci/mmol, 2 µCi/mL) was added to each well, and cultures were incubated at 37°C for 30 min. Following leucine incorporation, the cells were rinsed twice with HBSS and lysed with 100 µL of 0.1 N NaOH. The cell lysate was adsorbed on to filter disks and the proteins were precipitated with trichloroacetic acid and successive rinses of ethanol-acetone and acetone, respectively. The disks were dried, and incorporation of [^{3}H]-leucine was measured by liquid scintillation counting. The final values represent the mean of three separate wells.

5-Azidoresorcinol (4). To a solution of 5 g (28 mmol) of 4[17] in 125 mL of H_2O and 12.5 mL of conc HCl at 0°C was added a solution of 1.97 g (28 mmol) of $NaNO_2$ in 12.5 mL of H_2O over a 5 min period. The mixture was stirred for 10 min, and a solution of 2.13 g (33 mmol) of NaN_3 in 12.5 mL of H_2O was added. This solution was stirred an additional 40 min at 0°C and extracted with EtOAc. The crude product was chromatographed on silica gel using 1:2 EtOAc-hexane to afford 2.23 g (52%) of 4 as pale yellow crystals: mp 110-111°C; IR (KBr) 3300, 2097 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.07 (d, J=1 Hz, 2, ArH), 6.20 (t, J=1 Hz, 1, ArH), 8.53 (br s, 2, OH).

Anal. Calcd. for $C_6H_5O_2N_3$: C, 47.69; H, 3.33. Found: C, 47.70; H, 3.39.

Methyl (3-Azido-5-methoxyphenoxy)acetate (5a). To 436 mg (18.2 mmol, 1.1 eq) of NaH in 4 mL of anhydrous DMF was added 2.50 g (16.5 mmol) of 4 in 10 mL of anhydrous DMF. This mixture was stirred for 1 h at 25°C under nitrogen. To this solution was added 1.60 mL (16.0 mmol, 0.97 eq) of $BrCH_2CO_2CH_3$, and this mixture was stirred at 25°C for 30 min. An additional portion of 0.8 mL (8 mmol, 0.48 eq) of $BrCH_2CO_2CH_3$, and the solution was stirred for 30 min. The product was acidified to pH 4 using 5N HCl and diluted with EtOAc. The product was chromatographed on silica gel using 1:9 EtOAc-CH_2Cl_2 to afford 1.23 g (35%) of methyl (3-azido-5-hydroxyphenoxy)acetate: mp 118-120°C; IR (TF) 3400, 2100, 1750 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 3.70 (s, 3, OCH_3), 4.66 (s, 2, OCH_2), 6.10-6.28 (m, 3, ArH), 8.65 (br s, 1, OH); m/z calcd. for $C_9H_9N_3O_4$ 223.0594, found 223.0593.

To 1 g (4.48 mmol) of methyl (3-azido-5-hydroxyphenoxy)acetate in 10 mL of anhydrous DME was added 1.27 mL (13.5 mmol, 3 eq) of $(CH_3)_2SO_4$ and 3.09 g (22.4 mmol, 5 eq) of K_2CO_3. The solution was stirred for 2 h at 80°C under nitrogen, filtered, and extracted with EtOAc. The crude product was chromatographed on silica gel using 2:3 EtOAc-hexane to afford 0.95 g (90%) of 5a: mp 69-71°C; IR (KBr) 2120, 1755 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 3.70 (s, 3, OCH_3), 3.77 (s, 3, OCH_3) 4.73 (s, 2, OCH_2), 6.20-6.37 (m, 3, ArH); m/z calcd.

for $C_{10}H_{11}N_3O_4$ 237.0750, found 237.0751.

Methyl 4-(3-Azido-5-methoxyphenoxy)butyrate (5b). The procedure described for the preparation of 5a was repeated using 524 mg (21.8 mmol) of NaH, 3.0 g (19.9 mmol) of 4, 3.5 mL (16.0 mmol) of $Br(CH_2)_3CO_2CH_3$ to afford, after chromatography on silica gel using 1:9 EtOAc-CH_2Cl_2, 1.83 g (37%) of methyl 4-(3-azido-5-hydroxyphenoxy)butyrate: mp 75-78°C; IR (TF) 3400, 2120, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.00-2.30 (m, 2, CH_2), 2.43 (t, J=6 Hz, 2, CH_2), 3.36 (t, J=6 Hz, 2, CH_2), 3.63 (s, 3, OCH_3), 6.03-6.43 (m, 3, ArH), 6.66 (s, 1, OH); m/z calcd for $C_{11}H_{13}N_3O_4$ 251.0906, found 251.0904.

The procedure desribed for the preparation of 5a was repeated using 258 mg (1.03 mmol) of methyl 4-(3-azido-5-hydroxyphenoxy)butyrate, 244 mg (92.6 mmol) of $(CH_3)_2SO_4$, and 710 mg (5.14 mmol) of K_2CO_3 to afford, after chromatography on silica gel using 1:2 EtOAc-hexane, 254 mg (93%) of 5b: IR (TF) 2100, 1760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.08 (p, J=6 Hz, 2, CH_2), 2.52 (t, J=6 Hz, 2, CH_2), 3.70 (s, 3, OCH_3), 3.78 (s, 3, OCH_3), 3.98 (t, J=6 Hz, 2, CH_2), 6.17-6.24 (m, 3, ArH); m/z calcd for $C_{12}H_{15}N_3O_4$ 265.1064, found 265.1064.

(3-Azido-5-methoxyphenoxy)acetic Acid (6a). To 450 mg (1.90 mmol) of 5a was added 280 mg (5.10 mmol, 2.7 eq) of KOH in 14 mL of 1:6 aq ethanol. The solution was stirred at 25°C for 1 h, diluted with EtOAc, acidified to pH 4, and extracted with EtOAc. The crude product was recrystallized from EtOAc-hexane to afford 390 mg (92%) of 6a: dp 136-138°C; IR (KBr) 3600-2500, 2120, 1700 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.77 (s, 3, OCH_3), 4.73 (s, 2, OCH_2), 6.25 (d, J=1 Hz, 2, ArH), 6.35 (t, J=1 Hz, 1, ArH); m/z calcd for $C_9H_9N_3O_4$ 223.0593, found 223.0563.

4-(3-Azido-5-methoxyphenoxy)butyric Acid (6b). The procedure described for the preparation of 6a was repeated using 254 mg (0.958 mmol) of 5b and 268 mg of KOH in 5 mL of 6:1 aq ethanol to afford 156 mg (65%) of 6b: dp 94-96°C; IR (TF) 3800-2400, 2200, 1760 cm^{-1}; ^1H NMR ($CDCl_3$, DMSO-d_6) δ 2.03-2.16 (m, 2, CH_2), 2.52 (t, J=6 Hz, 2, CH_2), 3.77 (s, 3, OCH_3), 3.97 (t, J=6 Hz, 2, CH_2), 6.15-6.24 (m, 3, ArH); m/z calcd for $C_{11}H_{13}N_3O_4$ 251.0906, found 251.0904.

3-Azido-5-methoxybenzoyl Azide (10). To a solution of 1.74 g (10.4 mmol, 1.0 eq) of 9 in 50 mL of 2M HCl at 0-5°C was added a cold solution of 863 mg (12.5 mmol, 1.2 eq) of $NaNO_2$ in 2 mL of H_2O. The solution was stirred for 30 min and filtered. The cold filtrate was treated with 745 mg (11.5 mmol, 1.1 eq) of NaN_3 in 5 mL of H_2O. The mixture was stirred for 20 min and extracted with EtOAc. The solvent was evaporated to afford 1.7 g (85%) of 3-azido-5-methoxybenzoic acid: dp 164-166°C; IR (KBr) 3600-2200, 2100, 1695, 1595 cm^{-1}; ^1H NMR ($CDCl_3$, DMSO-d_6) δ 3.8 (s, 3, OCH_3), 6.68 (m, 1, C-2 ArH), 7.3 (m, 2, C-4, 6 ArH).

Anal. Calcd. for $C_8H_7N_3O_3$: C, 49.75; H, 3.65. Found: C, 49.63; H, 3.70.

The procedure described by Palmere and Conley[18] was repeated using 2.78 g (14.4 mmol, 1.0 eq) of 3-azido-5-methoxybenzoic acid in 8 mL of H_2O and sufficient acetone to obtain a clear solution. To this solution at 0°C was added 1.6 g (15.9 mmol, 1.1 eq) of Et_3N in 20 mL of acetone followed by 2.04 g (1.8 mL, 18.8 mmol, 1.3 eq) of $ClCO_2Et$ in 10 mL of acetone. The mixture was stirred for 30 min, and 1.4 g (21.7 mmol, 1.5 eq) of NaN_3 in 5 mL of H_2O was added. The mixture was stirred for 1 h, poured into H_2O at 0°C, and extracted with EtOAc. The solvent was evaporated to afford 2.93 g (93%) of 10: mp 52-56°C; IR (KBr) 2150, 2100, 1690, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.8 (s, 3, OCH_3), 6.72 (m, 1, C-2 ArH), 7.26 (m, 2, C-4 and 6 ArH); m/z calcd for $C_8H_6N_6O_2$ 218.0552, found 218.0551.

N-(3-Azido-5-methoxyphenyl) N'-(Carboxymethyl) Urea (11). The procedure of Palmere and Conley[18] was repeated by refluxing 2.83 g (13.0 mmol) of 10 in 100 mL of toluene for 20 h to afford 2.3 g of crude isocyanate: IR (TF) 2250, 2100 cm^{-1}. The procedure of Kurzer and Powell[19] was repeated using a solution of 300 mg (4.0 mmol, 2.0 eq) of glycine in 1 mL of H_2O, 3 mL of 2M NaOH at 15°C, and 380 mg (2.0 mmol, 1.0 eq) of isocyanate. The mixture was stirred for 30 min, and filtered. The product was precipitated by the slow addition of 1M HCl, and extracted with EtOAc. The solvent was evaporated to afford 253 mg (48%) of 11: dp 172-174°C; IR (KBr) 3700-2200, 2110, 1750 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.77 (s, 3, OCH_3), 3.98 (m, 2, CH_2), 6.14 (br s, 1, NH), 6.21 (m, 1, C-2 ArH), 6.92 (m, 1, C-4 ArH), 6.98 (m, 1, C-6 ArH), 8.40 (br s, 1, ArNH).

Anal. Calcd. for $C_{10}H_{11}N_5O_4$: C, 45.29; H, 4.18. Found: C, 45.35; H, 4.20.

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 15-Acetate (12). The procedure described by Pathre[11b] was repeated using 220 mg (0.55 mmol) of 1 in 30 mL of CH_3OH and 50 mL of 10% NaOAc in 1:4 CH_3OH-H_2O, which was adjusted to pH 9.5 using 0.1 M NaOH. After stirring for 5 h at 25°C, the mixture was concentrated, diluted with water, and extracted with EtOAc. The crude product was chromatographed on silica gel using 1:1 EtOAc-hexane to afford 124 mg (64%) of 12: IR (KBr) 3500, 1725, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 (s, 3, C-14 CH_3), 1.73 (s, 3, C-16 CH_3), 1.75-2.05 (m, 4, C-7, 8 CH_2), 2.07 (s, 3, $OCOCH_3$), 2.76 and 3.04 (AB q, J=3.9 Hz, 2, C-13 CH_2), 3.63 (d, J=4.9 Hz, 1, C-2 CH), 3.88 and 4.20 (AB q, J=12.3 Hz, C-15 CH_2), 3.96 (d, J=5 Hz, 1, C-11 CH), 4.20-4.30 (partially hidden m, 2, C-3, C-4 CH), 5.50 (br d, J=4.4 Hz, 1, C-10 vinylic H).

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 15-Acetate 4β-(N-(3-Azido-5-methoxyanilino)carboxy)glycinate (13). To 33 mg (0.1 mmol, 1.0 eq) of 12[11b] in 1 mL of anhydrous THF was added 35 mg of 11, 40 mg (0.15 mmol, 1.5 eq) of DCC, and 11 mg (0.09 mmol, 0.6 eq) of DMAP. The mixture was stirred at 25°C for 72 h, concentrated, and chromotographed first on silica gel using EtOAc to afford a mixture of C-3 and C-4 esters and then (using medium pressure chromotography) on silica gel to afford 7.0 mg (12%) of 13: ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3, C-14 CH_3), 1.69 (s, 3, C-16 CH_3), 2.02 (s, 3, $OCOCH_3$), 2.82 and 3.09 (AB q, 2, J=3.9 Hz, C-13 CH_2), 3.71 (s, 3, OCH_3), 3.72-4.15 (m, 7, C-2 CH, C-15 CH_2, C-3 CH, C-11 CH, and $COCH_2NH$), 5.10 (m, 1, C-4 CH), 5.45 (br d, J=5Hz, 1, C-10 vinylic H), 5.97 (m, 1, CH_2NH), 6.17 (m, 1, ArH), 6.61 (m, 1, ArH), 6.75 (m, 1, ArH), 7.63 (br m, 1, ArNH); m/z (FAB) calcd for $C_{27}H_{34}N_5O_9$ ($M+H^+$) 572.2357, found 572.2335.

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 4β,15-Diacetate 3α-Tetrahydropyranyl Acetal (14). The procedure of Roush[13] was repeated using 100 mg (0.27 mmol, 1.0 eq) of 1, 51 mg (0.6 mmol, 2.2 eq) of dihydropyran, and 14 mg of PPTS in 1.5 mL of CH_2Cl_2 at 25°C for 12 h. The crude product was chromatographed on silica gel using 1:1 EtOAc-hexane to afford 123 mg (100%) of 14: mp 89-92°C (lit[9c] mp 93-94°C); IR (KBr) 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (s, 3, C-14 CH_3), 1.74 (br s, 3, C-16 CH_3), 1.50-2.06 (m, 4, C-7, 8 CH_2), 2.08 and 2.12 (two s, 3, $OCOCH_3$), 2.79 and 3.05 (AB q, J=4 Hz, 2, C-13 CH_2), 3.52 (br d, J=8 Hz, 1, C-2 CH), 3.79-4.40 (m, 5, C-15 CH_2, C-3 CH, CH_2O of THP), 4.79 (d, J=6 Hz, 1, C-4 CH), 5.50 (br s, 1, CH of THP), 5.68 (br d, J=12 Hz, 1, C-10 vinylic H).

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 15-Acetate 3α-Tetrahydropyranyl Acetal (15). The procedure of Pathre[11b] was repeated using 123 mg (0.27 mmol, 1.0 eq) of 14[13] and 40 mL of 10% solution of NaOAc in 4:1 CH_3OH-H_2O, which was adjusted to pH 9.5 using 1.0 M NaOH, to afford, after chromatography on silica gel using 1:1 EtOAc-hexane, 52 mg (47%) of 15: IR (TF) 3440, 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 and 0.85 (two s, 3, C-14 CH_3), 1.72 (s, 3, vinylic CH_3), 2.059 and 2.062 (two s, 3, C-15 $OCOCH_3$), 2.74-3.04 (m, 2, C-13 CH_2), 4.97 (m, 1, C-4 CHOH), 5.50 (br d, J=6 Hz, 1, C-10 vinylic H).

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 15-Acetate 4β-(3-Azido-5-methoxyphenoxy)acetate (16). The procedure described for the preparation of 13 was repeated using 36 mg (0.091 mmol, 1.0 eq) of 15, 28 mg (0.136 mmol, 1.5 eq) of DCC, and 30 mg (0.136 mmol, 1.5 eq) of 6a in 3.0 mL of THF to afford, after chromatography on silica gel in 3:7 EtOAc-hexane, 17 mg (31%) of 16: ^{1}H NMR ($CDCl_3$) δ 1.73 (s, 3, vinylic CH_3), 2.06 (s, 3, $OCOCH_3$), 3.79 (s, 3, OCH_3), 4.73 (s, 2, $COCH_2O$), 5.48 (m, 1, C-10 vinylic H), 6.18-6.30 (m, 3, ArH); m/z (FAB) calcd for $C_{31}H_{39}N_3O_9$+H$^+$ 614.2715, found 614.2714.

3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol 4β,15-Diacetate (17). To 100 mg (0.214 mmol, 1 eq) of 1 in 0.5 mL of anhydrous DMF was added 96 mg (0.65 mmol, 3 eq) of t-BuMe$_2$SiCl,[14] and 73 mg (1.07 mmol, 5 eq) of imidazole. The solution was stirred at 25°C for 18 h and extracted with EtOAc. The crude product was chromatographed on silica gel using 2:3 EtOAc-hexane to give 133 mg (100%) of 17: IR (TF) 1755 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3, C-14 CH_3), 0.84 (s, 9, $C(CH_3)_3$), 1.70 (s, 3, C-16 CH_3), 2.00 and 2.03 (two s, 3, $COCH_3$), 2,70 and 2.97 (AB q, J=4.5 Hz, 2, C-13 CH_2), 3.90-4.30 (m, 5, C-2, C-3, C-11, C-15 CH and CH_2), 5.36 (d, J=5 Hz, 1, C-10 CH), 5.49 (d, J=3, 1, C-4 CH).

Anal. Calcd. for $C_{25}H_{40}O_7Si$: C, 62.47; H, 8.39. Found: C, 62.24; H, 8.24.

3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol 15-Acetate (18) and 3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol (21). The procedure of Pathre[11] was repeated using 53 mg (0.11 mmol, 1.0 eq) of 17 and 16 mL of 10% NaOAc in 4:1 CH_3OH-H_2O, which was adjusted to pH 9.5 using 1.0 M NaOH, to afford, after chromatography on silica gel using 1:1 EtOAc-hexane, 21 mg (31%) of 17 and 26 mg (53%) of 18: mp 110-113°C; IR (TF) 3380, 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.10 and 0.13 (two s, 6, $Si(CH_3)_2$), 0.80 (s, 3, C-14 CH_3), 0.92 (s, 9, $C(CH_3)_3$), 1.71 (br s, 3, C-16 CH_3), 1.65-2.04 (m, 4, C-7 and C-8 CH_2), 2.06 (s, 3, OAc), 2.73 and 2.99 (AB q, J=4 Hz, C-13 CH_2), 3.49 (d, J=5 Hz, 1, C-2 CH), 3.90 and 4.15 (AB q, J=12 Hz, 2, C-15 CH_2), 4.02-4.22 (m, 3, C-3, C-11, C-4 CH).

Anal. Calcd. for $C_{23}H_{38}O_6Si$: C, 62.98; H, 8.73. Found: C, 63.05; H, 8.78.

In addition, 6 mg (14%) of 21 was isolated: mp 189-190°C; IR (KBr) 3500 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.10 and 0.13 (two s, 6, $Si(CH_3)_2$), 0.88 (s, 3, C-14 CH_3), 0.93 (s, 9, $C(CH_3)_3$), 1.73 (s, 3, C-10 CH_3), 1.90-2.15 (m, 4, C-7 and C-8 CH_2), 2.73 and 2.99 (AB q, J=4 Hz, 2, C-13 CH_2), 3.47 (d, J=5 Hz, 1, C-2 CH), 3.53 and 3.77 (AB q, J=12 Hz, 2, C-15 CH_2), 4.00 (d, J=5 Hz, 1, C-11 CH), 4.12 (dd, J=3, 6 Hz, 1, C-3 CH), 4.28 (d, J=2 Hz, 1, C-4 CH), and 5.45 (br d, J=5 Hz, 1, C-10 CH).

Anal. Calcd. for $C_{21}H_{36}O_5Si$: C, 63.59; H, 9.15. Found: C, 63.50; H, 9.18.

3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol 15-Acetate 4β-(3-Azido-5-methoxyphenoxy)acetate (19a). The procedure described for the preparation of 13 was repeated using 103 mg (0.24 mmol, 1 eq) of 18, and 52.6 mg (0.24 mmol, 1 eq) of 6a, 56 mg (0.27 mmol, 1.15 eq) of

DCC, and 6.7 mg (0.047 mmol, 0.2 eq) of 4-(pyrrolidino)pyridine in CH_2Cl_2 to afford, after chromatography on silica gel using 1:4 EtOAc-hexane, 134 mg (89%) of 19a: IR (TF) 2120, 1770, 1750, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.06 and 0.08 (two s, 6, $Si(CH_3)_2$), 0.75 (s, 3, C-14 CH_3), 0.91 (s, 9, $C(CH_3)_3$), 1.72 (s, 3, C-16 CH_3), 1.86-2.03 (m, 4, C-7 and C-8 CH_2), 2.06 (s, 3, $OCOCH_3$), 2.77 and 3.04 (AB q, J=4 Hz, 2, C-13 CH_2), 3.54 (d, J=5 Hz, 1, C-2 CH), 3.76 (s, 3, OCH_3), 4.08 and 4.22 (AB q, J=12 Hz, 2, C-15 CH_2), 4.22 (m , 1, C-11 CH partially hidden by C-15 CH_2), 4.31 (dd, J = 3, 5 Hz, C-3 CH), 4.65 (s, 2, $COCH_2O$), 5.48 (br d, J=6 Hz, 1, C-10 CH), 5.73 (d, J=3 Hz, 1, C-4 CH), 6.40, 6.48, 6.56 (three t, J=2 Hz, 3, ArH).

3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol 15-Acetate 4β-(4-(3-Azido-5-methoxyphenoxy))butyrate (19b). The procedure described for the preparation of 13 was repeated using 18 mg (0.041 mmol, 1 eq) of 18, and 10.3 mg (0.049 mmol, 1.2 eq) of 6b, 10.1 mg (0.049 mmol, 1.2 eq) of DCC, and 1.2 mg (0.008 mmol, 0.2 eq) of 4-(pyrrolidino)pyridine in CH_2Cl_2 to afford, after chromatography on silica gel using 3:7 EtOAc-hexane, 27.1 mg (98%) of 19b: IR (TF) 2100, 1740, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.04 and 0.06 (two s, 6, $Si(CH_3)_2$), 0.70 (s, 3, C-14 CH_3), 0.89 (s, 9, $SiC(CH_3)_3$), 1.72 (s, 3, C-16 CH_3), 2.06 (s, 3, $OCOCH_3$), 2.00-2.18 (partially hidden m, 2, $OCH_2CH_2CH_2CO$), 2.59 (t, J=6.5 Hz, 2, $OCH_2CH_2CH_2CO$), 2.86 and 3.03 (AB q, J=4 Hz, 2, C-13 CH_2), 3.53 (d, J=5 Hz, 1, C-2 CH), 3.79 (s, 3, OCH_3), 3.99 (t, J=6.5 Hz, 2, $OCH_2CH_2CH_2CO$), 4.08 and 4.30 (AB q, J=12 Hz, 2, C-15 CH_2), 4.21 (d, J=5 Hz, 1, C-11 CH), 4.30 (partially hidden m, 1, C-3 CH), 5.48 (d, J=6 Hz, 1, C-10 CH), 5.59-5.63 (m, 1, C-4 CH), 6.20 (d, J=2 Hz, 2, ArH), 6.26 (m, 1, ArH); m/z (FAB) calcd for $C_{34}H_{49}N_3O_9SiLi$ (M^++Li) 678.3398, found 678.3382.

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 15-Acetate 4β-(3-Azido-5-methoxyphenoxy)acetate (20a). To a solution of 10 mg (0.155 mmol, 1 eq) of 19 in 200 µl of THF was added 14.8 µl of 1M (0.015 mmol, 0.95 eq) $(n\text{-}Bu)_4NF$ in THF. The solution was stirred at 25°C for 15 min, diluted with H_2O, and extracted with EtOAc. The product was chromatographed on silica gel using 1:1 EtOAc-hexane to give 5.7 mg (70%) of 20a: IR (TF) 3440, 2105, 1740, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (s, 3, C-14 CH_3), 1.75 (br s, 3, C-16 CH_3), 1.95-2.10 (m, 4, C-7 and C-8), 2.06 (s, 3, $OCOCH_3$), 2.80 and 3.09 (AB q, 1, C-13 CH_2), 3.06 (br d, J=3 Hz, 1, OH), 3.73 (d, J=5 Hz, 1, C-2 CH), 3.79 (s, 3, OCH_3), 4.01 and 4.15 (AB q, J=12 Hz, 2, C-15 CH_2), 4.15 (m, partially hidden, 1, C-11 CH), 4.24 (dd, J=3, 5 Hz, C-3 CH_2), 4.71 (s, 2, $COCH_2O$), 5.36 (d, J=3 Hz, 2, C-4 CH_2), 5.55 (br d, J=4 Hz, 1, C-10 vinylic H), 6.39, 6.51, 6.58 (three t, J=2 Hz, 3, ArH); m/z calcd for $C_{26}H_{31}N_3O_9$-N_2 501.1999, found 501.2003.

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 15-Acetate 4β-(4-(3-Azido-5-methoxyphenoxy))butyrate (20b). The procedure described for the preparation of 20a was repeated using 27.1 mg (0.040 mmol, 1 eq) of µmol) of 19b and 40.3 µL of 1M (0.040 mmol, 1 eq) of $(n\text{-}Bu)_4NF$ in THF to afford, after chromatography on silica gel using 1:1 EtOAc-hexane, 16.2 mg (72%) of 20b: IR (TF) 3440, 2120, 1750, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 (s, 3, C-14 CH_3), 1.73 (s, 3, C16-CH_3), 1.80-2.14 (m, 4, C-7 and C-8 CH_2), 2.04 (s, 3, CH_3C=O), 1.96-2.14 (m, 2, CH_2CH_2O), 2.63 (t, J=6.5 Hz, 2, CH_2C=O), 2.78 and 3.07 (AB q, J=2 Hz, C-13 CH_2), 3.22 (br s, 1, OH), 3.71 (d, J=2.5 Hz, 1, C-2 CH), 3.78 (s, 3, OCH_3), 3.94-4.03 (m, 3, CH_2O and C-15 CH), 4.10-4.21 (m, 3, C-15 CH, C-11 CH, and C-3 CH), 5.16 (d, J=2.9 Hz, 1, C-4 CH), 5.55 (d, J=5 Hz, 1, C-10 CH), 6.19 (d, J=2 Hz, 2, ArH) and 6.25 (t, J=2 Hz, 1, ArH); m/z (FAB) calcd for $C_{28}H_{35}N_3O_9$+H^+ 558.2453, found 558.2437.

3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol (26) from Diol 21. To a solution of 47 mg (0.12 mmol, 1 eq) of 21 in 1.5 mL of anhydrous C_6H_6 was added 15 mg (0.14 mmol, 1.2 eq) of N-acetylimidazole. To this solution was added 3.6 mg (24 µmol, 0.2 eq) of DBU. The solution was stirred at 25°C for 24 h, concentrated, and chromatographed on silica gel using 1:2 EtOAc-hexane to afford 32 mg (62%) of 26: IR (TF) 3475, 1730 cm^{-1}; ^1HNMR ($CDCl_3$) δ 0.06 (s, 3, $SiCH_3$), 0.08 (s, 3, $SiCH_3$), 0.75 (s, 3, C-14 CH_3), 0.91 (s, 9, $C(CH_3)_3$), 1.72 (s, 3, C-16 CH_3), 1.98-2.06 (m, 4, C-7 and C-8 CH_2), 2.16 (s, 3, $OCOCH_3$), 2.75 and 3.01 (AB q, J=4 Hz, 2, C-13 CH_2), 2.80 (br s, 1, OH), 3.54 (d, J=5 Hz, 1, C-2 CH), 3.65 and 3.88 (AB q, J=13 Hz, 2, C-15 CH_2), 4.31-4.36 (m, 2, C-11 and C3 CH), 5.54 (d, J=6 Hz, 1, C-10 CH), 5.75 (d, J=3 Hz, 1, C-4 CH).

Anal. Calcd. for $C_{23}H_{38}O_6Si$: C, 62.98; H, 8.73. Found: C, 62.91; H, 8.74.

3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol (26) from Diacetate 17. To a solution of 92 mg (0.192 mmol) of 17 in 5 mL of anhydrous DME was added 20 mg (0.84 mmol, 4.4 eq) of LiOH and 34 µL (0.84 mmol, 4.4 eq) of CH_3OH.[16] The solution was stirred at 25°C for 6 days and extracted with EtOAc. The product was chromatographed on silica gel using 1:2 EtOAc-hexane to afford 33.5 mg (36%) of 17 and 37.5 mg (45%, 64% based on recovered 17) of 26 having spectral data identical with that described above.

3α-tert-Butyldimethylsilyloxy-12,13-epoxytrichothec-9-ene-4β,15-diol 4β,15-Di-(3-azido-5-methoxyphenoxy)acetate (24). The procedure described for the preparation of 13 was repeated using 16 mg (0.04 mmol) of 21, 17.9 mg (0.08 mmol, 1 eq) of 7, 22 mg (0.106 mmol, 2.6 eq) of DCC (in two portions in which the second portion was added after 1.5 h), and 2.2 mg (0.015 mmol, 0.4

eq) of 4-pyrrolidinopyridine in CH_2Cl_2 to afford, after chromatography on silica gel using 3:7 EtOAc-hexane, 18.9 mg (58%) of 24: IR (TF) 1770, 1750, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.06 and 0.08 (two s, 6, $Si(CH_3)_2$), 0.68 (s, 3, C-14 CH_3), 0.91 (s, 9, $SiC(CH_3)_3$), 1.72 (s, 3, C-13 CH_3), 1.82-2.06 (m, 4, C-7 and C-8 CH_2), 2.73 and 3.02 (AB q, J=4 Hz, 2, C-13 CH_2), 3.53 (d, J=5 Hz, 1, C-2 CH), 3.77 (s, 6, OCH_3), 4.15 and 4.26 (AB q, J=12 Hz, 2, C-15 CH_2), 4.09-4.38 (m, 2, C-3 and C-11 CH, partially hidden), 4.60-4.65 (m, 4, $COCH_2O$), 5.46 (br d, J=5 Hz, 1, C-10 vinylic H), 5.62 (d, J=2 Hz, 1, C-4 CH), and 6.14-6.58 (m, 6, ArH); m/z (FAB) calcd for $C_{39}H_{50}N_6O_{11}SiLi$ (M^++Li) 813.3467, found 813.3460.

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 4β,15-Di-(3-azido-5-methoxyphenoxy)acetate (25). To a solution of 18.9 mg (0.024 mmol) of 24 in 1 mL of anhydrous THF was added 23 μl of 1M (6.2 mg, 0.024 mmol, 1 eq) $(n\text{-}Bu)_4NF$ in THF. The solution was stirred for 15 min and chromatographed on silica gel using 1:1 EtOAc-hexane to afford 10.9 mg (68%) of 25: IR (TF) 3440, 2110, 1760, and 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (s, 3, C-14 CH_3), 1.72 (s, 3, C-16 CH_3), 1.60-2.02 (m, 4, C-7 and C-8 CH_2), 2.75 and 3.07 (AB q, J=4 Hz, 2, C-13 CH_2), 3.71 (d, J=5 Hz, 1, C-2 CH), 3.77 (s, 3, OCH_3), 3.78 (s, 3, OCH_3) 4.07-4.29 (m, 4, C-3 and C-11 CH, C-15 CH_2), 4.60 (s, 2, OCH_2CO), 4.69 ((s, 2, OCH_2CO), 5.35 (d, J=3 Hz, 1, C-4 CH), 5.52 (q, J=1 Hz, 5 Hz, C-10 CH), 6.15 (t, J=2 Hz, 1, ArH), 6.17 (t, J=2 Hz, 1, ArH), 6.23 (m, 4, ArH); m/z (FAB) does not show M+H$^+$ but does show fragment corresponding to the conversion of one N_3 group to NH_2: calcd for $C_{33}H_{36}N_6O_{11}$-N_2+H_2+H^+ 667.2617, found 667.2636.

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 4β-Acetate 15-(3-Azido-5-methoxyphenoxy)acetate (27). The procedure described for the preparation of 13 was repeated using 30.4 mg (69 μmol) of 26, 15.5 mg (69 μmol, 1 eq) of 6a, 17.5 mg (83 μmol, 1.2 eq) of DCC, and 2.2 mg (15 μmol, 0.2 eq) of 4-(pyrrolidino)pyridine in CH_2Cl_2 to afford, after chromatography on silica gel using 1:1 EtOAc-hexane, 28.1 mg (63%) of the C-3 TBS ether of 27: ^1H NMR ($CDCl_3$) δ 0.70 (s, 6, $SiCH_3$), 0.63 (s, 3, C-14 CH_3), 0.90 (s, 9, $C(CH_3)_3$), 1.72 (s, 3, C-16 CH_3), 1.80-2.00 (m, 4, C-7 and C-8 CH_2), 2.09 (s, 3, $OCOCH_3$), 2.70-2.99 (AB q, J=3 Hz, 2, C-13 CH_2), 3.49 (d, J=3 Hz, 1, C-2 CH), 3.78 (s, 3, OCH_3), 4.06-4.33 (m, 4, C-3 and C-11 CH, C-15 CH_2), 4.63 (d, J=1.5 Hz, 2, OCH_2), 5.37-5.53 (m, 2, C-10 and C-4 CH), 6.13-6.27 (m, 3, ArH); m/z (FAB) calcd for $C_{32}H_{45}N_3O_9SiLi$ (M^++Li) 650.3086, found 650.3069.

To a solution of 28.1 mg (44 μmol) of the above product in 2 mL of anhydrous THF was added 43 μL of 1M (11.4 mg, 44 μmol, 1 eq) $(n\text{-}Bu)_4NF$ in THF. The solution was stirred for 15 min and chromatographed on silica gel using 1:1 EtOAc-hexane to afford 18.6 mg (81%) of 27a: IR (TF) 3460, 2110, 1740, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (s, 3, C-14 CH_3), 1.72 (s, 3, C-16 CH_3), 1.80-2.04 (m, 4, C-7 and C-8 CH_2), 2.14 (s, 3, OAc), 2.75 and 3.06 (AB q, J=4 Hz, 2, C-13 CH_2), 3.15 (br s, 1, OH), 3.69 (d, J=5 Hz, 1, C$_2$CH), 3.74 (s, 3, OCH_3), 4.1 and 4.29 (AB q, J=13 Hz, 2, C-15 CH_2), 4.10 (m, partially hidden, 1, C-11 CH), 4.20 (q, J=3 Hz, 4 Hz, 1, C-3 CH), 4.62 (s, 2, OCH_2CO), 5.13 (d, J=3 Hz, 1, C-4 CH), 5.52 (d, J=6 Hz, 1, C-10 CH), 6.16 (t, J=2 Hz, 1, ArH), 6.23 (d, J=2 Hz, 2, ArH); m/z calcd for $C_{26}H_{31}N_3O_9$-N_2 502.2077, found 502.2071.

12,13-Epoxytrichothec-9-ene-3α,4β,15-triol 4β-Acetate 15-(3-Azido-4-iodo-5-methoxyphenoxy)acetate (28). To a solution of 10.7 mg (20.2 μmol) of 27 in 150 μL of CH_3CN and 30 μL of H_2O was added 12.7 mg (84 μmol, 4.2 eq) of NaI and 9.6 μL (85 μmol, 4.2 eq) of t-BuOCl. The solution was stirred at 25°C for 14 h. The crude product was chromatographed on silica gel in 2:3 hexane-EtOAc to afford 3 mg (23%) of 28: IR (TF) 3460, 2130, 1725, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (s, 3, C-14 CH_3), 1.74 (s, 3, C-16 CH_3), 1.80-2.02 (m, 4, C-7 and C-8 CH_2), 2.17 (s, 3, $OCOCH_3$), 2.76 and 3.08 (AB q, J=4 Hz, 2, C-13 CH_2), 3.08 (partially hidden s, 1, OH), 3.72 (d, J=5 Hz, 1, C-2 CH), 3.93 (s, 3, OCH_3), 4.08,and 4.35 (AB q, J=12 Hz, 2, C-15 CH_2), 4.08 (partially hidden d, 1, C-11 CH), 4.75 (s, 2, OCH_2CO), 5.08 (d, J=3 Hz, 1, C-4 CH), 5.54 (br d, J=5 Hz, 1, C-10 CH), 6.10 (s, 1, ArH), 6.28 (s, 1, ArH); m/z (FAB) calcd for $C_{26}H_{30}N_3O_9Li$ (M+Li$^+$) 662.1200, found 662.1190.

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