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NEOCARZINOSTATIN CHROMOPHORE: PRESENCE OF A HIGHLY STRAINED ETHER RING AND ITS REACTION WITH MERCAPTAN AND SODIUM BOROHYDRIDE

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Spectroscopic evidence suggests the presence of a highly strained ether ring (Fig. 1) (possibly an epoxide) in the C_{12}-subunit of the previously determined partial structure 2a (Fig. 2) of the major neocarzinostatin chromophore (NCS-Chrom A) which completes assignment of all the oxygens in the molecule. The main product from mercaptan treatment suggests opening of the ether ring involving the addition of one molecule of mercaptan as well as reduction of the C_{12}-substructure, whereas a parallel two-step reduction occurs on $NaBH_4$ treatment. Both reactions occur with rearrangement of the C_{12}-substructure and the implication for the mechanism of action of NCS-Chrom A in DNA strand scission activity is discussed. The evidence suggests a downward revision of the molecular formula for NCS-Chrom A as well as minor components B and C by two protons.

We recently proposed (1,2) partial structure 2a (Fig. 2) for the major biologically active non-protein chromophore, NCS-Chrom A‡ ($C_{35}H_{35}NO_{12}$, MW 661) of the antitumor protein antibiotic neocarzinostatin (NCS) (3,4), consisting of cyclic carbonate (1,3-dioxolan-2-one), 2-hydroxy-5-methoxy-7-methyl-1-naphthoate and 2,6-dideoxy-2-methylaminogalactose moieties linked to an incompletely defined C_{12}-substructural unit.

‡Abbreviations: NCS, neocarzinostatin; NCS-Chrom A, B and C, neocarzinostatin non-protein chromophore components A, B and C; HPLC, high pressure liquid chromatography; MS, mass spectra; HRMS, high resolution mass spectra; EI, electron impact; FD, field desorption; FAB, fast atom bombardment; MW, molecular weight; TMS, $-SiC_3H_8$; BSTFA, bis-trimethylsilyl-trifluoroacetamide; NMR, nuclear magnetic resonance; (ppm), chemical shift in parts per million; J(Hz), coupling constant in Hertz; J_{gem}, geminal coupling constant; J_{vic}, vicinal coupling constant; $^{1,2,3}J_{^{13}C\text{-}^{1}H}$, one-, two- and three-bond ^{13}C-^{1}H coupling constants; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; APT, attached proton test; SFORD, single-frequency off-resonance decoupling.

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Minor components NCS-Chrom B 2b ($C_{34}H_{37}NO_{11}$, MW 635) and C 2c ($C_{36}H_{39}NO_{13}$, MW 693) (Fig. 2) were found to be solvolysis products of A involving opening of the cyclic carbonate ring (5). The molecular formulae were derived by HRMS of the trimethylsilylated derivatives of the mercaptan reaction products, either CH_3SH or $HSCH_2CO_2CH_3$, on the assumption that no reduction accompanies the addition (2). No vaporisable product for MS analysis either by electron impact (EI) or field desorption (FD) was obtained in the absence of mercaptan treatment.

We now have strong ^1H and ^{13}C NMR as well as further MS (FAB) evidence to suggest that addition as well as reduction is implicated in the reaction of NCS-Chrom A with mercaptan, necessitating a downward revision of the molecular formula for NCS-Chrom A by two hydrogens and similarly for B and C. Furthermore, we wish to elaborate on the reactive moiety in the C_{12}-subunit, the main source of instability of the chromophore (1,4,6) and its modification by mercaptan and sodium borohydride, reagents required for the chromophore's ability to cause DNA damage in vitro (4,7,8,9). In particular, we present evidence in support of a strained ether ring (Fig. 1), possibly an epoxide, in addition to the earlier assigned oxygen functions.

MATERIALS AND METHODS

NCS (clinical ampules, Kayaku Antibotics, Tokyo, containing 1.3 mg NCS per ampule, 90 and 10%, respectively, of NCS-Chrom A and B) was stored frozen in 0.015M sodium acetate, pH 5. Deuteromethylthioglycolate ($DSCH_2CO_2CH_3$) was prepared by diluting $HSCH_2CO_2CH_3$ to 0.5M in CH_3OD at RT, at least 12 hours before using. The chromophore was extracted with 0.1M acetic acid in methanol from drug after dialysis against 0.1M acetic acid followed by lyophilization. For the reactions with deuterated reagents, the salt-free NCS was lyophilized from CD_3CO_2D in D_2O, and the chromophore extracted with 0.1M CD_3CO_2D in CH_3OD. The dialysis, extraction and reactions were done at 4°C in the dark. NCS-Chrom A and B, and the reaction products with $NaBH_4/NaBD_4$ and mercaptan, were separated by HPLC on a Waters μ Bondapak C_{18} column (3.9 mm x 30 cm) (1) in 0.01M ammonium acetate, pH 4, with a gradient of

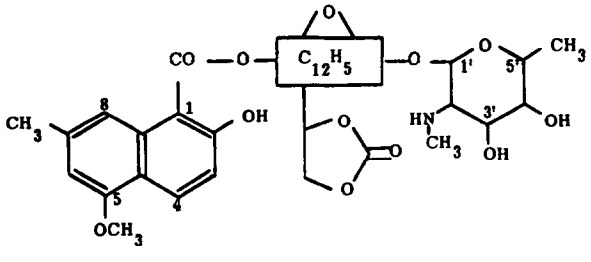


Figure 1. Newly proposed partial structure of NCS-Chrom A incorporating the remaining oxygen function in the C_{12}-subunit as an ether ring (possibly an epoxide).

50 and 80% methanol (4,10,11). The reaction of NCS-Chrom in 0.1M acetic acid with solid $NaBH_4$ was followed by HPLC, observing the loss of the original chromophore (retention time, 63 min) and generation of the major product (retention time, 42 min) (11). A similar product from NCS-Chrom B elutes at 41 min. The methylthioglycolate product was prepared by addition of the thioglycolate to an acidic methanol suspension of NCS. Excess thioglycolate was removed by concentration under reduced pressure and lyophilization. The reaction products were extracted with methanol and separated by HPLC. The major mercaptan product of NCS-Chrom A elutes at 46.5 min (11). An identical mercaptan product was isolated from the reaction of mercaptan with a protein-free chromophore extract in acetic acid in methanol. The HPLC profile of the mercaptan reaction products is very similar to that of the $NaBH_4$ reaction products, with a significant minor peak, 8.5 min, identified by MS as the naphthoic acid of NCS-Chrom (11). An estimated 30% yield of the major $NaBH_4$ or mercaptan product was isolated by HPLC. The fractions separated by HPLC were collected on ice, the methanol removed by evaporation under reduced pressure, the sample frozen and lyophilized. The lyophilized samples were redissolved in a minimum volume of appropriate protonated or deuterated solvents for MS or NMR.

^1H NMR spectra of the $NaBH_4/NaBD_4$ and mercaptan products were obtained in CD_3CO_2D at 25°C on a Varian SC-300 spectrometer. ^{13}C NMR spectra of NCS-Chrom (90% A, 10% B) (and a confirmatory ^1H NMR spectrum (see Table 1)), were obtained on a Bruker WM-250 instrument at 0°C in a 10 mm tube. The sample was obtained by extraction of 200 mg dialyzed clinical NCS with CD_3CO_2D (20 ml), concentration to 2 ml and dilution to 2.5 ml with CD_3OD. EI-MS data were obtained on a Finnigan MAT-212 mass spectrometer after trimethylsilylation with BSTFA in dry pyridine (see Table 2). FAB spectra were obtained on a Varian model 731 instrument fitted with an Ion Tech saddle field gun operating at 8 Kv/0.1 ma. The samples were dissolved in a glycerol matrix containing p-toluenesulfonic acid on a sample probe of our own design with 70° angle of incidence for the ion beam.

RESULTS AND DISCUSSION

We previously showed that trimethylsilylation after mercaptan treatment of NCS-Chrom A gives a tetra-TMS derivative implicating four exchangeable protons, none of which were at the time assumed to be formed in the mercaptan treatment (2). Two of these are associated with the sugar ring (the sterically hindered secondary amine is not silylated), and one each for the naphthoate and C_{12}-substructure (see 2a, Fig. 2). This leaves six carbon-bound protons in the C_{12}-subunit, only five of which have so far been observed in ^1H NMR spectra. We now have obtained ^{13}C NMR data on the unstable chromophore in CD_3CO_2D:CD_3OD (4:1) at 0°C which confirms the carbon count of 35 and a non-active proton count of 29 from both 'gated' as well as APT spectra (12). All of the protonated carbons (Table 1) were readily assigned on the basis of SFORD experiments and comparison with chemical shift data of model compounds (13,14). Assignments of the quaternary carbons of the naphthoic acid ring were made by comparison of chemical shift and two- and three-bond ^{13}C-^1H coupling constant data with those of methyl salicylate and naphthalene derivatives (14), allowing the remaining five methine and eight quaternary carbons to be assigned to the C_{12}-subunit and the carbonyl carbon of the cyclic carbonate.

Table 1. ^{1}H and ^{13}C NMR Assignments of NCS-Chrom A in CD_3CO_2D-CD_3OD (4:1) at 0°C

Assignment	^{1}H (ppm)[a,b]	^{13}C (ppm)	$^{1}J_{^{13}C-^{1}H}$ [b]	$^{2,3}J_{^{13}C-^{1}H}$ [b]
S5'-CH_3	1.26 d (6.5)	16.4 q[d]	127	sharp s
N7-CH_3	2.64 s	~20 obsc[c]		
S2'-NCH_3	3.02 s	33.1 q[d]	145	sharp s
M	4.17 d (~1)	55.0 d[d]	197	sharp s
N5-OCH_3	3.83 s	55.6 q[d]	145	sharp s
S2'	3.72 dd (3.5,10.5)	59.2 d[e,h]	~147	v.br.m
M	-	63.6 s	-	v.br.m
CC-CH_2	4.55 dd (5, 9)	68.0 dd[d]	155	s
	4.76 dd (8, 9)		160	
S3'	4.26 dd (2.5, 10.5)	68.0 d[d]	143	br.m
S5'	4.10 q (6.5)	68.9 d[e,h]	141	br.s
S4'	3.90 d (2.5)	72.2 d[d]	148	br.s
CC-CH	4.93 dd (5, 8)	76.0 d[d]	162	sharp s
M	5.12 br.s	81.3 d[d]	151	br.m
M	6.24 br.s	82.7 d[d]	157	br.m
M	-	87.3 s	-	br.s
M	-	90.5 s[f,h]	-	br.s
S1'	5.77 d (3.5)	95.3 d[f,h]	169	br.s
M	-	97.6 s[i]	-	d (4.5)
N4a	-	99.7 s[d]	-	br.m
N8	7.81 d (2)	104.2 d[d]	161	d (4)
M	-	105.9 s[f]	-	br.s
M	5.82 br.s	106.5 d[g,i]	172	d (4)
N3	7.02 d (9)	116.4 d[g,i]	165	sharp s
N6	6.90 d (2)	117.7 d[i,j]	161	v.br.m
N1	-	124.1 s[d]	-	br.m
M	-	129.9 s[d]	-	br.m
N4	8.10 d (9)	134.0 d[i]	159	sharp
N8a	-	135.0 s[i]	-	sharp d (7)
N7	-	138.3 s[d]	-	br.m
M	6.82 br.s	139.6 d[k]	~174	br.s
CC-C=O	-	155.9 s[i]	-	v.br.m
N5	-	160.4 s[d]	-	br.m
M	-	160.7 s[i,j]	-	br.s
N2	-	164.5 s[j]	-	sharp d (11)
N1-CO_2R	-	172.6 s[d]	-	sharp s

Chemical shifts (δ) are given in ppm downfield of internal tetramethylsilane. Abbreviations: N = naphthoate, S = aminosugar, M = C_{12}-subunit, CC = cyclic carbonate, s = singlet, d = doublet, q = quartet, m = multiplet, v = very, br = broad, obsc = obscured

a The ^{1}H NMR chemical shifts for NCS-Chrom A in CD_3CO_2D in Table 1 of Ref. 2 should be displaced by 0.09 ppm.

b Coupling constants are given in Hz

c obscured by the CD_3CO_2D methyl peak

d assigned unequivocally on the basis of SFORD

e,f,g Cannot be distinguished on the basis of SFORD

h assigned by comparison with 2-amino-2-deoxy-galactose hydrochloride (13)

i assigned by comparison with naphthalene derivatives (14)

j assigned by comparison with methyl salicylate

k assigned by comparison with the value (155.9 ppm, CD_3CO_2D) in propylene carbonate

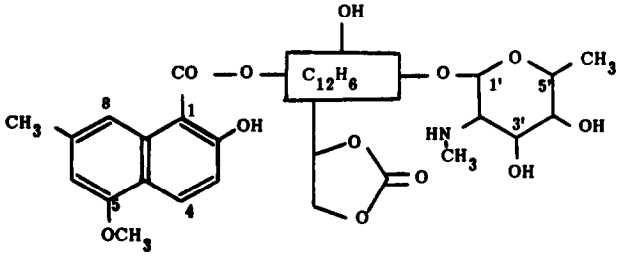
Noteworthy is the methine at 55.0 ppm, correlating with the proton doublet ($J \simeq 1$ Hz) at δ 4.17, which is characterized by an unusually large ^{13}C-^{1}H coupling constant of 197 Hz. This is strongly suggestive of a strained epoxide on the basis of

Table 2. Critical HRMS data on trimethylsilyl derivatives of $NaBH_4$ reduction product of NCS-Chrom A and B[a]

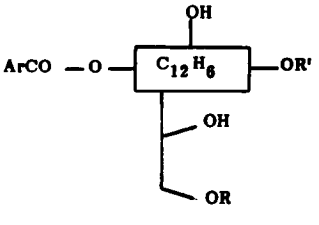
Assignment	A	B
M	$C_{35}H_{37}NO_{12}.TMS_4$ (Found 951)	$C_{34}H_{39}NO_{11}.TMS_6$ (Found 1069.4916) (Calc 1069.4895)
$M-CH_3$	$C_{34}H_{34}NO_{12}.TMS_{3-2/3}$ (Found 936.3643) (Calc 936.3662)	$C_{33}H_{36}NO_{11}.TMS_{5-2/3}$ (Found 1054)
$M-C_{13}H_{12}O_4.TMS_1$	$C_{22}H_{25}NO_8.TMS_3$ (Found 647.2744) (Calc 647.2766)	$C_{21}H_{27}NO_7.TMS_5$ (Found 765.3785) (Calc 765.3764)
$M-C_7H_{14}NO_3.TMS_2$	$C_{28}H_{23}O_9.TMS_2$ (Found 631.2168) (Calc 631.2184)	$C_{27}H_{25}O_8.TMS_4$ (Found 749.3198) (Calc 749.3182)

[a] The composition of each fragment ion is given with the observed and calculated high resolution mass values in brackets. The mass ions at m/z 951 and 1054 were too weak to be measured. The number of trimethylsilyl groups was verified by comparison with perdeutero-TMS derivatives.

chemical shift (15,16) and by comparison of the $^1J_{^{13}C-^1H}$ of the normal value of 178 Hz for the methine in styrene epoxide (δ 53.3 ppm) and a value of 199 Hz for the strained epoxide 3 (δ57.8 ppm). Although an oxetane ring cannot be completely ruled out for lack of suitable data, further discussion will be made in terms of an epoxide. This interpretation accounts for the last oxygen atom of the chromophore and suggests



2a, NCS-Chrom A



2b, R = H , NCS-Chrom B

2c, R = $COCH_3$, NCS-Chrom C

Figure 2. Previously proposed (1) partial structures of the major (NCS-Chrom A) and minor (NCS-Chrom B and C) active chromophores of NCS.

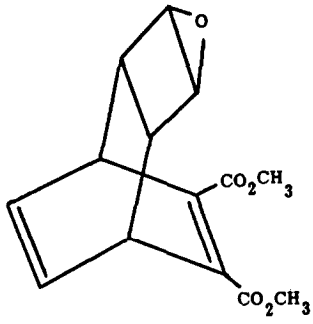


Figure 3.

a revision of the elemental composition of $C_{35}H_{35}NO_{12}$ (MW 661) to $C_{35}H_{33}NO_{12}$ (MW 659), consistent with the number of non-exchangeable protons in the NMR spectra. Contrary to the earlier tentative assumption (2), treatment with a mercaptan therefore appears to generate a hydroxy group.

We were able to investigate this hypothesis further by examination of the chromophore directly by Fast Atom Bombardment (FAB), a technique recently acquired in our laboratories (17). Under protonating conditions, positive-ion FAB of a sample of chromophore freshly extracted from clinical NCS resulted in a strong peak at m/z 660 for NCS-Chrom A and a smaller one at m/z 634 corresponding to the 10% minor NCS-Chrom B component (1). These values, indicative of molecular weights of 659 and 633 for NCS-Chrom A and B, respectively, are strongly supportive of the NMR data.

Sodium borohydride reduction product. This reaction is of interest as the requirement for mercaptan in the in vitro DNA scission activity of NCS-Chrom A can be replaced by $NaBH_4$ as was reported for native NCS (7). Changes in the absorption and fluorescence spectral properties resulting from borohydride treatment are similar to those observed for mercaptan and have been discussed elsewhere (11). HPLC analysis of the reaction products NCS-Chrom A with $NaBH_4$ (11) shows an intensely fluorescent major peak eluting at 42 min, showing a strong ion at m/z 664 by FAB under protonating conditions. The product accepts four TMS groups resulting in molecular ions by EI-MS of m/z 951 (^{2}H-TMS analog: m/z 987). As indicated in Table 2, strong ions appearing at m/z 647 (^{2}H-TMS analog: m/z 674) and m/z 631 (^{2}H-TMS analog: m/z 649) correspond to the loss of the naphthoic acid fragment as $C_{13}H_{12}O_4 \cdot TMS_1$ and aminosugar moiety

as $C_7H_{14}NO_3 \cdot TMS_2$, respectively. Loss of the sugar fragment was not observed for the mercaptan-treated product (2). Similar to the mercaptan spectra are the presence of characteristic ions for the naphthoic acid residue at m/z 287 and 214 and for the aminosugar moiety at m/z 320, 304, 175 and 145. The corresponding $NaBD_4$ product shows a strong ion three mass units higher at m/z 667 by FAB which is supported by EI-MS of the trimethylsilylated derivative having strong peaks at m/z 954 (M^+), 939 (M^+-Me), 650 and 634 (^{2}H-TMS analogs: m/z 990, 975, 677 and 652, respectively). The naphthoate and aminosugar fragments are therefore unmodified which is supported by unchanged fragment ions at m/z 287 and 214 (naphthoate) and m/z 320, 304, 175 and 145 (aminosugar).

The addition of three carbon-bound protons to the C_{12}-substructure of NCS-Chrom A by $NaBH_4$ was corroborated by ^{1}H NMR comparison with that of A. Correlation between the C_{12}-subunit protons in the two spectra is difficult but it is clear that the three new resonances at δ6.01 br.s, 7.66s and 6.97d (5.5) are absent in the spectrum of the $NaBD_4$ product. All resonances of the naphthoate (18), the aminosugar and the cyclic carbonate moieties remain relatively unchanged.

From the addition of three carbon-bound protons to the C_{12}-substructure of NCS-Chrom A in the reaction with $NaBH_4$ and confirmed by $NaBD_4$, it is inferred that (a minimum of) four protons are added and that the MW must be 659 (or smaller). Reduction of a double or triple bond and opening of the ether ring generating one silylatable OH function, is consistent with the addition of four protons and a MW of 659 in agreement with the NMR and FAB data on the intact chromophore.

Analogous EI-MS data were obtained on the $NaBH_4/NaBD_4$ products of NCS-Chrom B. Both products accept six TMS groups resulting in molecular ions of m/z 1069/1072 (^{2}H-TMS analog: m/z 1123/1126). Loss of the naphthoic acid fragment as $C_{13}H_{12}O_4 \cdot TMS_1$ and aminosugar as $C_7H_{14}NO_3 \cdot TMS_2$ is indicated by strong peaks at m/z 765/768 (^{2}H-TMS analog: m/z 810/813) and m/z 749/752 (^{2}H-TMS analog: m/z 785/788), respectively (see Table 2). Characteristic ions for the naphthoic acid residue at m/z 287 and 214 and for the aminosugar moiety at m/z 320, 304, 175 and 145 are also present. The data support a MW of 633 instead of 635 (1) for NCS-Chrom B corresponding to the molecular formula $C_{34}H_{35}NO_{11}$.

Methylthioglycolate product of NCS-Chrom A. Comparison of the ^1H NMR spectra of the major products from $NaBH_4$ and $HSCH_2CO_2CH_3$ treatment of NCS-Chrom A in methanol show a remarkable similarity. The resonances of the naphthoate, aminosugar and cyclic carbonate moieties remain relatively unchanged (18) whereas two extra resonances are observed in the δ 4.0-8.5 region by comparison with NCS-Chrom A spectra. These findings are in contrast to those of Sheridan and Gupta (19) who suggest modification of the naphthoic acid ring. All five protons of the C_{12}-subunit in NCS-Chrom A occur as broad singlets whereas the mercaptan and borohydride spectra are characterized by two sharp singlets at δ7.52/7.42 and 7.83/7.66 and two sharp doublets (J=5.5 Hz) at δ6.42/6.35 and 7.01/6.97 which are coupled. In the corresponding $NaBD_4$ spectrum, the sharp singlet at δ7.66 and doublet at δ 6.97 are missing whereas the other doublet appears as a singlet as expected. The deuterothioglycolate spectrum shows the singlet at δ7.83 and doublet at δ 7.01 at reduced intensity and a new MeO singlet at δ 3.78 (the methylene absorption of the introduced reagent is obscured near δ 3.06). The integrated intensity of this MeO signal relative to the other three methyl groups in the same spectral region, is unequivocally shown in the solvent system CD_3CO_2D:C_6D_6 (3:1) to be 1:1:1:1. The ^1H NMR evidence therefore suggests the addition of one molecule of thioglycolate and two protons to the C_{12}-substructure, supporting a MW of 659 for NCS-Chrom A. In agreement with these findings are the previously reported (2) EI-MS data on the thioglycolate reaction product of NCS-Chrom A which resulted in ions at m/z 1055 and 951 corresponding to the compositions (excluding TMS groups) $C_{38}H_{41}NO_{14}S$ (M^+) and $C_{35}H_{37}NO_{12}$(M^+-S = $CHCO_2CH_3$), respectively (20).

The proposal of an ether ring in the C_{12}-subunit of NCS-Chrom A (as well as B and C) completes the assignment of all oxygens in the molecule (Fig. 1). In particular, we find no evidence for the presence of an aliphatic aromatic diacyl peroxide as previously proposed by Edo et al (21,22). Furthermore, no evidence of ^{18}O incorporation was obtained by MS analysis of chromophore extracted from NCS protein and treated with mercaptan in the presence of $^{18}O_2$. Opening of the ether ring with generation of a hydroxyl group and reduction of a double or triple bond readily account for the stoichiometry of the reactions with mercaptan and sodium borohydride. However, the

^{1}H NMR chemical shifts and couplings, especially of the protons newly introduced into the C_{12}-substructure, strongly suggest that a rearrangement accompanies the reduction/addition process (23). While the in vitro DNA scission activity of methanol-extracted NCS-Chrom is highly dependent on the presence of a mercaptan (4,7), the chromophore is rapidly inactivated by the mercaptan in the absence of DNA (6). The two-step reduction/addition reaction of NCS-Chrom A, implicating a total of three molecules of mercaptan, suggests a partial reduction or addition product, possibly with rearrangement, as the 'active' species, conceivably an activated cyclic ether. That reduction occurs first leaving the ether ring intact, would be consistent with the finding of Povirk and Goldberg (manuscript in preparation) that two molecules of mercaptan are utilized per molecule of NCS-Chrom A in the presence of DNA. Subsequent opening of the ether ring with another molecule of mercaptan, with or without rearrangement, in the absence of DNA, thus may lead to inactivation (25). Although mechanistically not equivalent, reductive activation involving a three-membered ring has precedence in the interaction of mitomycins with DNA where, after reduction, irreversible loss of activity upon opening of the aziridine ring has been observed (26).

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REFERENCES AND NOTES

1. Napier, M.A., Goldberg, I.H., Hensens, O.D., Dewey, R.S., Liesch, J.M. and Albers-Schönberg, G. (1981) Biochem. Biophys. Res. Commun. 100, 1703-1712.
2. Albers-Schönberg, G., Dewey, R.S., Hensens, O.D., Liesch, J.M., Napier, M.A. and Goldberg, I.H. (1980) Biochem. Biophys. Res. Commun. 95, 1351-1356.
3. Napier, M.A., Holmquist, B., Strydom, D.J. and Goldberg, I.H. (1979) Biochem. Biophys. Res. Commun. 89, 635-642.
4. Kappen, L.S., Napier, M.A. and Goldberg, I.H. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1970-1974.
5. NCS-Chrom B, though formally equivalent to the hydrolysis/decarboxylation product of NCS-Chrom A and present in all preparations of NCS studied, did not appear to derive from A during any of the isolation and chemical modification schemes (1).

6. Kappen, L.S. and Goldberg, I.H. (1980) Biochemistry 19, 4786-4790.
7. Goldberg, I.H., Hatayama, T., Kappen, L.S., Napier, M.A. and Povirk, L.F. (1981) "Protein antibiotics as DNA damaging agents" in "Molecular Actions and Targets for Cancer Chemotherapeutic Agents," Sartorelli, A.C., Bertino, J.R. and Lazo, J.S. (eds.). Second Annual Bristol-Myers Symposium in Cancer Research, Academic Press, New York pp. 163-191.
8. Povirk, L.E. and Goldberg, I.H. (1982) Proc. Natl. Acad. Sci. 79, 369-373.
9. Kappen, L.S., Goldberg, I.H. and Liesch, J.M. (1982) Proc. Natl. Acad. Sci. 79, 7444-7448.
10. Napier, M.A., Holmquist, B., Strydom, D.J. and Goldberg, I.H. (1981) Biochemistry 20, 5602-5608.
11. Napier, M.A. and Goldberg, I.H. (1983) Mol. Pharmacol. 23, 500-510.
12. Patt, S.L. and Shoolery, J.N. (1982) J. Magn. Reson. 46, 535-539.
13. Yamaoka, N., Usui, T., Sugiyama, H. and Seto, S. (1974) Chem. Pharm. Bull. 22, 2196-2200.
14. Breitmaier, E., Haas, G. and Voelter, W. (1979) "Atlas of Carbon-13 NMR Data," Heyden & Son, Philadelphia, Vol. 2.
15. Davies, S.G. and Whitham, G.H. (1975) J. Chem. Soc. Perkin II, 861-863.
16. Paulson, D.R., Tang, F.Y.N., Moran, G.F., Murray, A.S., Pelka, B.P. and Vasquez, E.M. (1975) J. Org. Chem. 40, 184-186.
17. Barber, M., Bordoli, R. S., Sedgwick, R.D. and Tyler, A.N. (1981) Nature 293, 270-275; Williams, D.H., Bradley, C., Bojesen, G., Santikarn, S. and Taylor, L.C.E. (1981) J. Am. Chem. Soc. 103, 5700-5704.
18. Only the N5-OMe singlet is significantly shifted to δ3.35 in the $NaBH_4$ product and believed to be due to long range shielding. Similarly, the resonance is found at δ3.27 in the thioglycolate product.
19. Sheridan, R.P. and Gupta, R.K. (1981) Intern. J. Quantum Chem: Quantum Biology Symp. 8, 331-346.
20. The reason for incomplete deuterium incorporation from deuterothioglycolate observed by ^{1}H NMR and EI-MS is under investigation.
21. Edo, K., Ito, M., Ishida, N., Koide, Y., Ito, A., Haga, M., Takahaski, T., Suzuki, Y. and Kusano, G. (1982) J. Antibiotics 35, 106-110.
22. In addition to our previous ^{1}H NMR evidence (1), the cyclic carbonate moiety is also well characterized by ^{13}C NMR. The data (see Table 1) compare favorably with those for propylene carbonate in CD_3CO_2D i.e. 72.1 br.t (155), 75.5 br.d (158) and 157.7 s ppm. The definitive assignment of the methine proton by SFORD allows the unequivocal assignments of J_{gem} = 9 and J_{vic} = 5,8 Hz. (see footnote 13 in Ref. 1).
23. Reduction of a disubstituted triple bond to a double bond, as the chemical shifts of the newly introduced protons suggest, appears attractive and is supported by ^{13}C NMR signals in the 80-100 ppm (24) range. The results indicate, however, that they are not adjacent as would be expected by simple 1,2-addition. Rearrangement is implicated regardless of whether 1,2- or perhaps 1,4-addition of a conjugated chromophore occurs.
24. Levy, G.C., Lichter, R.L. and Nelson, G.L. (1980) "Carbon-13 Nuclear Magnetic Resonance Spectroscopy", Wiley-Interscience, New York, 2nd edition, p. 92.
25. The unusual stability of the chromophore towards acid could be due in part to steric hindrance of the epoxide, even though highly strained, which may be alleviated in the activation step by mercaptan or sodium borohydride. Also, acid-catalyzed epoxide solvolysis in a second protonation of the molecule after protonation of the secondary amine function of the methylaminogalactose ring, may not be energetically favorable.
26. Iyer, V.N. and Szybalski (1964) Science 145, 55-58.