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SYNTHESIS AND DNA CLEAVING PROPERTIES OF HYBRID MOLECULES CONTAINING PROPARGYLIC SULFONES AND MINOR GROOVE BINDING LEXITROPSINS

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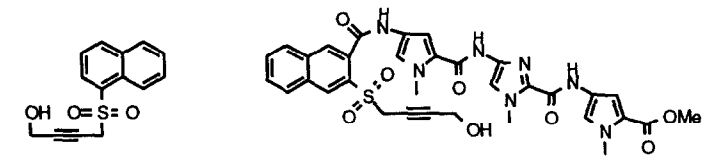
Abstract: *A series of hybrids incorporating propargylic sulfones and minor groove binding oligopeptide carriers* ***2-11*** *were synthesized and their abilities to cleave DNA have been demonstrated.*

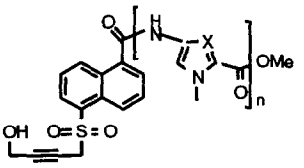
The enediynes are a rapidly emerging class of anticancer antibiotics derived from bacterial sources.[1] These molecules elicited extensive research activities in chemical, biochemical and biomedical circles and inspired the design of a number of novel molecular assemblies to probe and mimic their chemical and biological actions. Thus a new body of synthetic methodology and several novel synthetic strategies have already been devised to address the challenges posed by these molecules, and several new cleaving agents have been designed and synthesized.[2] Among these new molecules, propargylic sulfones such as compound **1** (Figure 1) were reported to be potent antitumor agents which cleave DNA in a pH-dependent fashion.[2n] To enhance the DNA binding and cutting ability and to impart base- and site- selectivities to these agents we have designed hybrid molecules containing the DNA cleaving moiety and sequence-specific lexitropsin carriers. In this communication we disclose a novel synthesis of a series of hybrids of propargylic sulfones and pyrrole and / or imidazole containing oligopeptides, derived from a class of antitumor antibiotics that preferentially bind in the minor groove of double-helical DNA at specific AT or GC regions respectively.[3]

Figure 1

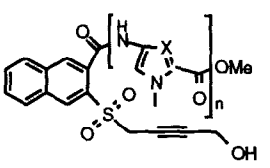


1

2



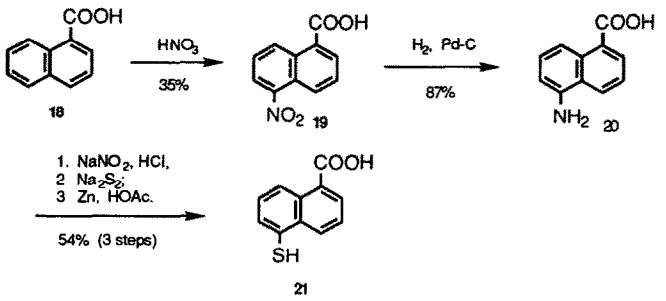
3 X = CH, n = 1
4 X = CH, n = 2
5 X = CH, n = 3
6 X = N, n = 1



7 X = CH, n = 1
8 X = CH, n = 2
9 X = CH, n = 3
10 X = N, n = 1
11 X = N, n = 2

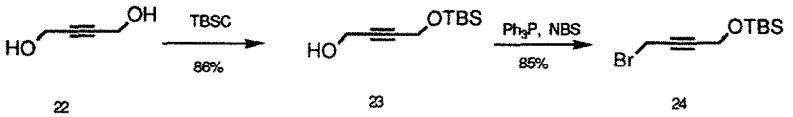
Syntheses of hybrid molecules. Naphthalene compound **21,** possessing two desired functions, is a suitable building block for the construction of the hybrids such as **3, 4, 5** and **6**. Synthesis of **21** was accomplished from the readily available 1-naphthoic acid **18** in 5 steps (Scheme I): The acid **18** was nitrated to afford a mixture of 8-nitro-1-naphthoic acid and 5-nitro-1-naphthoic acid **19,** which was separated by recrystallization (35% yield).[4] Catalytic hydrogenation of **19** with 10% Pd-C gave an excellent yield (87%) of amine **20**. The latter was converted to the key intermediate **21**[5] via 3 steps following the reported procedure[6] in 54% overall yield.

Scheme I



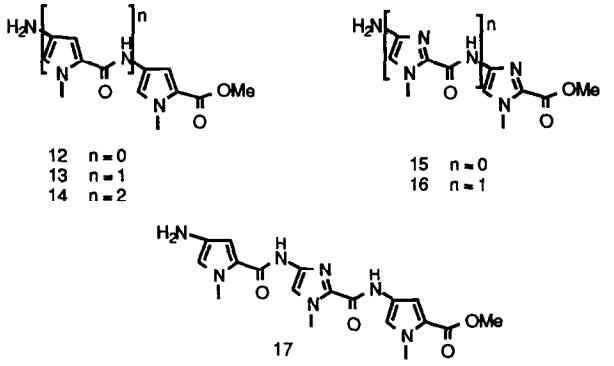
Slow addition of one equivalent of tert-butyldimethylsilyl chloride to **22** at room temperature provided the selectively silylated diol **23** (86% yield) (Scheme II). Treatment of **23** with triphenyl phosphine and N-bromosuccinimide (NBS)[7] led readily to the propargylic bromide **24** (85% yield) as a pale yellow oil. Although **23** can be stored in the refrigerator for several months without decomposition, the bromide **24** is relatively unstable and was used immediately after the preparation.

Scheme II



The final assembly of the desired hybrid molecules was achieved via a four-step pathway and is outlined in Scheme III. **21** was first condensed with the propargylic bromide **24** in the presence of triethylamine to give the appropriate propargylic sulfide **25** (76% yield). Oxidation of **25** with dioxirane[8] afforded the propargylic sulfone **26** in almost quantitative yield. The latter was then coupled with the pyrrole containing oligopeptide **12,** prepared from N-methyl pyrrole according to the method previously developed in this group[9] (figure 2), in the presence of 1-(3-dimethylaminopropyl)1, 3-ethylcarbodiimide hydrochloride (EDCI), leading to the hydroxy-protected hybrid **27** in a moderate yield of 52%. Desilylation of **27** with HF gave the final hybrid **3**[10] as an off-white solid in 79% yield after silica gel chromatography (eluent: petrolum ether to 30% ethyl acetate). Compounds **4-6** were thus prepared in reasonable yields bearing the respective oligopeptides **13-15.**[11] Similarly, starting from the commercially available 3-amino-2-naphthoic acid, compounds **7-11**[12] were synthesized bearing the oligopeptides **12-17** respectively.

Figure 2

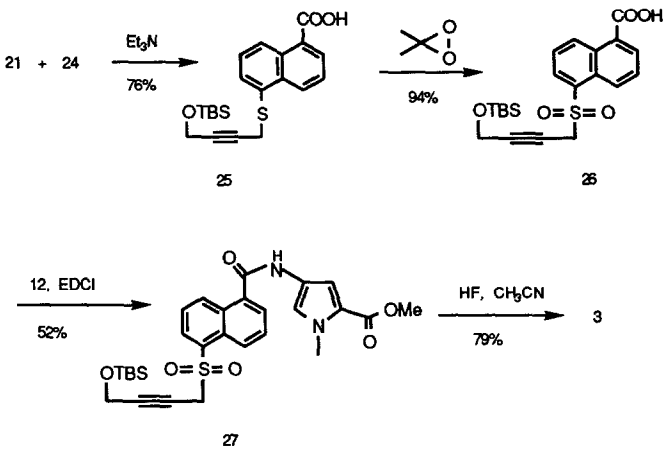


12 n = 0
13 n = 1
14 n = 2

15 n = 0
16 n = 1

17

Scheme III



25

26

27

DNA Cleavage Studies. DNA cleaving activities of the hybrids **2-11** on PM2 covalently closed circular (CCC) DNA were estimated by agarose gel electrophoresis. A single-strand break converted CCC DNA (Figure 3, form I) into the open circular (OC) DNA (form II). After electrophoresis each DNA band was visualized by ethidium bromide staining and densitometry. Compounds **3-6** (lane 4-7) exhibit high DNA cleaving activities, comparable with compound **1** (lane 3). Compounds **7-11, 2** (lane **9-11** and **14-16**) were comparatively less active. The results of the ethidium fluorescence assay, an independent quantitative method for the analysis of CCC DNA cleavage of these hybrids,[13a] are consistent with those of the gel study (Figure 4). In addition, relative binding constants of **1'** and representative **1'**, **5'** and **9'**, hydroxy-silylated **5** and **9**, were determined.[13b] It was found that **5** binds to calf thymus DNA more strongly than **9** (their binding constants are as follows: **1'**, 4.6×10^4 M^{-1}; **5'**, 7.2×10^5 M^{-1}; **9'**, 0.89×10^5 M^{-1}), which is in accord with the more

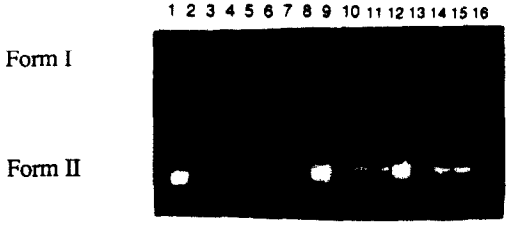


Figure 3. PM2-CC-DNA was incubated for 15 h at 37°C with the hybrids **2-11** (each at 2×10^{-5} M) in buffer (1 M Tris-HCl, pH 8.0) and analyzed by electrophoresis (1.0% agarose gel, ethidium bromide stain). Lanes 1, 8 and 12: Control PM2-CC-DNA; lanes 2 and 13: SI Nuclease on PM2-CC-DNA; lane 3: standard propargylic sulfone **1**; lanes 4-7: hybrids **3-6**; lanes 9-11: hybrids **7-9**; lanes 14-16: hybrids **11**, **2**, **10**, respectively; Form I = PM2-CC-DNA; Form II = OC-DNA (single strand cleavage)

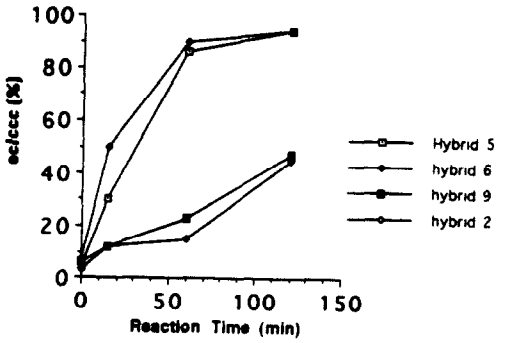


Figure 4. Single strand scission of PM2-CC-DNA by the hybrids **5**, **6**, **9**, and **2** each at a final concentration of 2×10^{-5} M. The reactions were performed in the same condition as described in Figure 3. OC/CC (%) was determined from the fluorescence readings before and after the denaturation at 96°C followed by rapid cooling.[13]

efficient DNA cleavage observed for compounds **3-6** compared with **7-11**. Further investigation to identify the site selectivity of the DNA cleavage of these hybrids and its relationship to cytotoxic potency is currently in progress and will be reported in due course.

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References and Notes

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5. For **21**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.25 (br s, 1H), 8.64 (br d, 1H, J = 8.5 Hz), 8.31 (br d, 1H, J = 8.5 Hz), 8.14 (d, 1H, J = 6.5 Hz), 7.72 (dd, 1H, J = 6.5, 1.2 Hz), 7.64 (t, 1H, J = 8.5 Hz),

7.50 (t, 1H, J = 8.5 Hz), 5.85 (br s, 1H); IR (CH_2Cl_2/MeOH Cast): 3054, 1673, 1609, 1585, 1568, 1296, 782 cm^{-1}; HRMS (EI): m/z calcd. for $C_{11}H_8O_2S$ (M^+): 204.0248. found: 204.0247.

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10. For **3**: ^1H NMR (DMSO-d_6, 300 MHz) δ 10.63 (s, 1H), 8.86 (d, 1H, J = 6.5 Hz), 8.59 (d, 1H, J = 6.5 Hz), 8.31 (dd, 1H, J = 5.5 , 2.0 Hz), 8.17 (s, 1H), 7.73-7.87 (m, 2H), 7.55 (d, 1H, J = 2.0 Hz), 6.88 (d, 1H, J = 2.0 Hz), 5.11 (t, 1H, J = 4.1 Hz), 4.53 (t, 2H, J = 2.0 Hz), 3.93 (dt, 2H, J = 4.1, 2.0 Hz), 3.89 (s, 3H), 3.74 (s, 3H): IR (CH_2Cl_2/MeOH Cast): 3361, 2941, 2901, 1699, 1643, 1575, 1576, 1531, 1452, 1317, 1288, 1131 cm^{-1}; HRMS (FAB): Calcd. for $C_{22}H_{21}N_2O_6S$ 441.1120. Found 441.1110 (M+H).
11. For **5**: ^1H NMR (DMSO-d_6, 300 MHz) δ 10.71 (s, 1H), 10.00 (s, 1H), 9.95 (s, 1H) 8.86-8.89 (m, 1H), 8.58 (d, 1H, J =8.2 Hz), 8.35 (d, 1H, J = 8.2 Hz), 7.72-7.80 (m, 3H), 7.49 (d, 1H, J = 2.0 Hz), 7.41 (d, 1H, J = 2.0 Hz), 7.38 (d, 1H. J = 2.0 Hz), 7.19-7.21 (m, 2H), 6.92 (d, 1H, J = 2.0 Hz), 5.20 (t, 1H, J = 5.5 Hz), 4.72 (s, 2H), 3.98 (d, 2H, J = 5.5 Hz), 3.95 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H) 3.75 (s, 3H); IR (CH_2Cl_2/MeOH Cast) : 3315, 3120, 2942, 1639, 1577, 1546, 1462, 1401, 1348, 1199, 1059 cm^{-1}; HRMS (FAB): Calcd. for $C_{34}H_{33}N_6O_8S$ 685.2080. Found 685.2052 (M+H).
12. For **9**: ^1H NMR (DMSO-d_6, 300 MHz) δ 10.85 (s, 1H), 10.10 (s, 1H), 9.94 (s, 1H), 8.72 (s, 1H), 8.34 (d, 1H, J = 6.8 Hz), 8.29 (s, 1H), 8.20 (d, 1H, J = 6.8 Hz), 7.77-7.90 (m, 2H), 7.48 (d, 1H, J = 2.0 Hz), 7.34 (d, 1H, J = 2.0 Hz), 7.27 (d, 1H, J = 2.0 Hz), 7.09 (d, 1H, J = 2.0 Hz), 7.05 (d, 1H, J = 2.0 Hz), 6.92 (d, 1H, J = 2.0 Hz), 5.22 (t, 2H, J = 6.4 Hz), 4.90 (s, 2H), 4.06 (m, 2H), 3.92 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.73 (s, 3H); IR (CH_2Cl_2/MeOH Cast): 3386, 3319, 2949, 1699, 1640, 1575, 1543, 1465, 1433, 1311, 1203 cm^{-1}; HRMS (FAB): Calcd. for $C_{34}H_{33}N_6O_8S$ 685.2080. Found 685.2054 (M+H).
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