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THE SYNTHESIS AND ANTITUMOR EVALUATION OF UNSYMMETRICAL BIS-IMIDES

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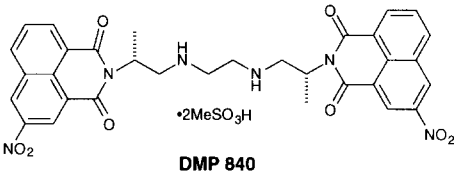
Abstract: Unsymmetrical *bis*-imides **1** were synthesized and evaluated as potential antitumor agents. These novel *bis*-imides were assessed using three criteria: in vitro cytotoxicity (L1210), in vitro DNA binding, and in vivo studies with human tumor xenografts in mice. These studies identified DMP 315 as a potent, water soluble antitumor agent.

Symmetrical *bis*-naphthalimides are effective agents against a variety of solid tumors.[1,2] From our program emerged DMP 840, which is a symmetrical *bis*-naphthalimide showing excellent activity[3] in vivo against several human tumor xenografts in mice. Because of this activity, DMP 840 was selected for further development and is currently in Phase II clinical trials.



DMP 840

DMP 840 is a DNA intercalator, which shows selective binding for GC rich fragments of DNA in vitro[4] as well as excellent growth inhibition in vitro.[5] From preliminary studies, DMP 840 appears to be a monointercalator.[4] However, at the present time, the mechanism of action for DMP 840 is still unclear.[4-6]

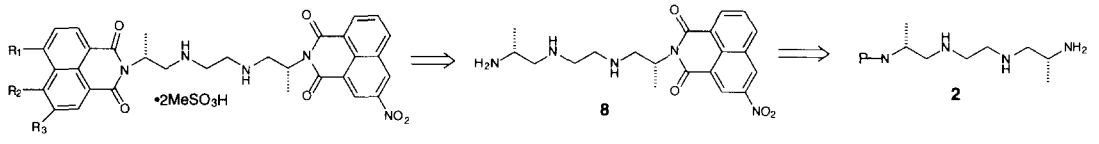
In this report, we describe our efforts directed toward a second generation *bis*-imide. The goal was to improve the potency as well as the physical properties. DMP 840 has a moderate water solubility (3.5 mg/mL), which provides limited flexibility in formulation and dosing.

As for the design of the second generation, we've used a working model featuring monointercalation of one chromophore into DNA as suggested above. We therefore surmised that the two chromophores had different functions. This suggested that unsymmetrical *bis*-imides could be more active, if one were able to optimize the functions separately. To pursue this approach, we required a synthetic route into the unsymmetrical *bis*-imides **1.**

Chemistry

These unsymmetrical compounds **1** contain a chiral, methyl substituted, tetraamine linker with different aromatic chromophores attached as imides on either end. Our strategy was to synthesize a differentially protected linker **2**, thereby allowing mono condensation of the constant 3-nitronaphthalic chromophore first, followed by a deprotection of "P" to give the mono-imide **8** (Scheme 1). A second condensation with a different chromophore would then afford unsymmetrical *bis*-imides **1**. However, chiral tetraamines that are differentially protected like **2** are rare,[7] and therefore, we developed a novel approach to the linker from amino acids based on a N-sulfonamide protection.

Scheme 1

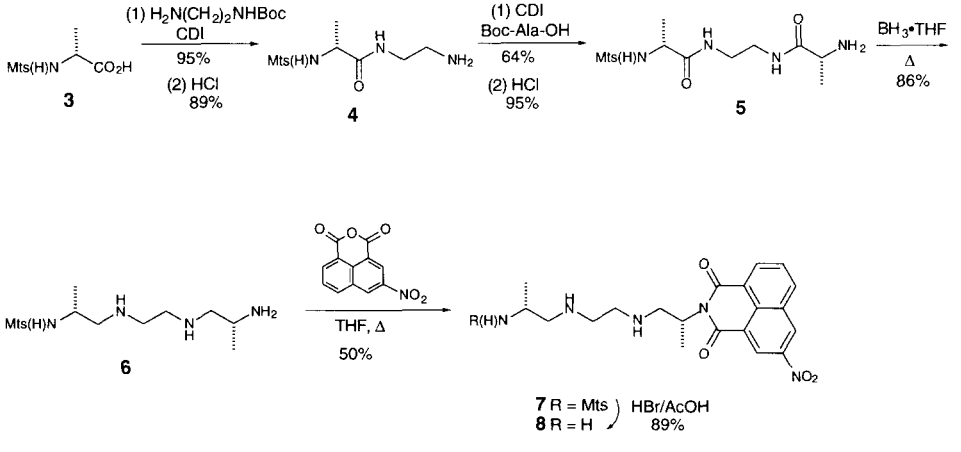


1 $R_3 \neq NO_2$ when $R_1, R_2 = H$

Linker Synthesis

As shown in Scheme 2, the route commenced with the coupling of N-mesitylenesulfonyl-D-alanine **3**[8] with N-*tert*-butoxycarbonyl-1,2-ethanediamine[9] to afford, after N-Boc deprotection, the amide **4**.[10] A second coupling with N-Boc-D-Alanine and subsequent deprotection yielded the diamide **5**. The critical amide reduction with BH_3•THF proceeded smoothly to give the triamine **6**, with the N-mesitylenesulfonyl still intact . This triamine **6** was then condensed with 3-nitronaphthalic anhydride to provide the imide **7**. With one chromophore in place, the sulfonamide had to be removed without destruction of the nitro containing imide. After some experimentation, it was found that HBr/AcOH easily removed the mesityl of **7** to give the monoimide **8** in high yield and purity. With the synthesis of linker **8** completed, we needed access to a variety of novel chromophores for the second condensation.

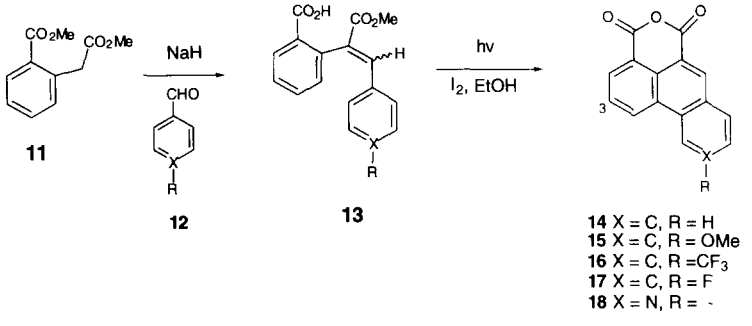
Scheme 2

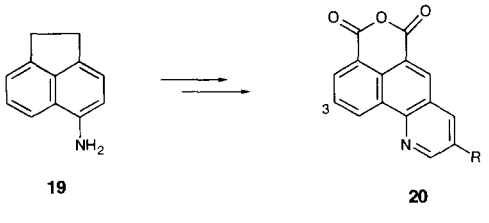


Anhydride Synthesis

As shown above, entry to the unsymmetrical *bis*-imides required a supply of aromatic anhydrides for the second condensation reaction. In order to drive the intercalation toward a single chromophore of the unsymmetrical *bis*-imide, we investigated polycyclic phenanthrene and aza-phenanthrene moieties that contain more π-density than the naphthalic group of DMP 840. Many of the corresponding anhydrides are novel, and were synthesized by the ring closure methods described below. A photochemical ring closure[11] was used to synthesize the phenanthrene anhydride **14**, the 6-substituted phenanthrene anhydrides **15-17**, and the 6-azaphenanthrene anhydride **18**; however, all proceeded in poor to moderate yield (20-40%). As shown in Scheme 3, the required stilbene **13** was synthesized in good yield (65-81%) via a Stobbe condensation between dimethyl homophthalate **11** and selected benzaldehydes **12**. The 5-azaphenathrene anhydrides **20**[12] were synthesized by a known method using 5-aminoacenaphthene **19**. Each of the azaphenanthrene anhydrides could be selectively nitrated (HNO_3/H_2SO_4) at the 3-position.

Scheme 3

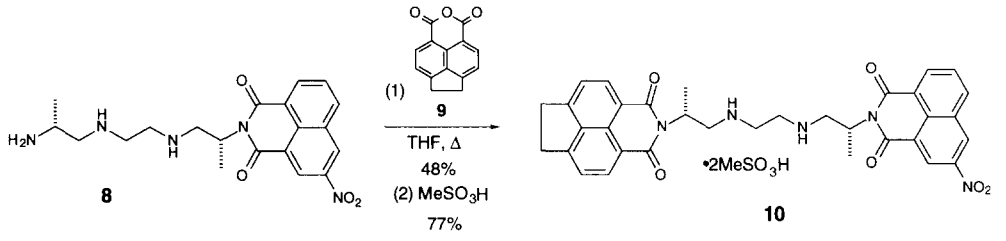




Synthesis of Unsymmetrical *Bis*-imides

With a supply of anhydrides and the key mono-imide **8** in hand, the stage was set for the synthesis of the unsymmetrical *bis*-imides. This was accommplished by heating the linker **8** with the desired anhydride in dioxane, THF, or DMF for an extented period of time. For example, the condensation of monoimide **8** with the ethano-bridged anhydride **9**[13] in refluxing THF gave the unsymmetrical *bis*-imide **10** as it's salt after treatment with methanesulfonic acid (Scheme 4). Some of the unsymmetrical *bis*-imides produced in this fashion are shown in Table I and II.

Scheme 4

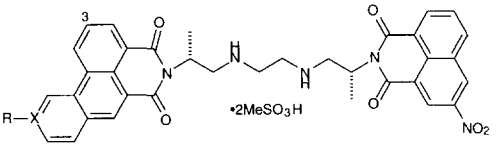


Results and Discussion

In Vitro Results

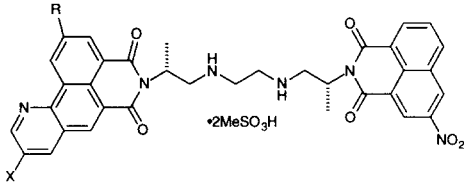
The unsymmetrical compounds **1** were evaluated in vitro using two criteria: DNA binding (ethidium bromide displacement) and growth inhibition (L1210 murine leukemia).[4,5] As mentioned, DMP 840 binds selectively to GC rich DNA, and therefore we wanted to examine the unsymmetrical compounds in this regard. As shown in Table I, incorporation of a phenanthrene chromophore, as in compound **22**, deters efficient binding to DNA. Electron donating (compound **23**) and withdrawing groups (compounds **24** and **25**) were installed at the 6-position of the phenanthrene, but neither helped the DNA binding or the L1210 activity. However, changing to a 6-azaphenathrene, as in DMP 315, restored the DNA binding affinity and some of the GC selectivity. More importantly, DMP 315 displayed excellent L1210 activity (0.035 μM), which is on par with DMP 840. With most symmetrical compounds, we have found the 3-nitro groups to be critical for in vitro activity. Therefore, we nitrated the azaphenathrene to give compound **26**, but were surprised to find a decrease in the L1210 potency. The azaphenanthrene nitrogen of DMP 315 can be moved around the ring as in compound **27** with a 5-azaphenanthrene. In this case, nitration of the 5-azaphenathrene ring gave compound **28**, which does restore the L1210 activity. The 5-azaphenathrene compounds also showed activity with a 7-methyl (compound **29**) and a 7-fluoro (compound **30**) substitution. These results indicate that substitution at the 6-position disturbs the DNA interaction in this class. On the other hand, the azaphenanthrenes have a planar, electron-rich chromophore, which does not disrupt the DNA binding. With these in vitro active compounds in hand, we selected several for further study in vivo.

Table I. In Vitro Results



Comp. #	X	R	dA•dT IC_{50} (μM)	dG•dC IC_{50} (μM)	AT/GC	IC_{50} L1210 (μM)
10	Scheme 4		1.57	0.11	14	0.075
22	C	H	1.02	0.97	1.1	0.42
23	C	OMe	0.80	0.13	6.2	0.25
24	C	CF_3	2.55	0.17	15	0.45
25	C	F	1.51	0.11	14	0.31
DMP 315	N	-	1.2	0.088	14	0.035
26	N	- (3-NO_2)	0.88	0.062	14	0.051
DMP 840			1.00	0.040	25	0.034

Table II. In Vitro Results



Comp. #	X	R	dA•dT IC_{50} (μM)	dG•dC IC_{50} (μM)	AT/GC	IC_{50} L1210 (μM)
27	H	H	1.08	0.073	15	0.25
28	H	NO_2	1.57	0.085	19	0.024
29	Me	NO_2	0.61	0.073	8.4	0.041
30	F	H	1.42	0.059	24	0.064
DMP 840			1.00	0.040	25	0.034

In Vivo Results

As shown in Table III, the compounds were tested at their MTD (maximum tolerated dose) versus three human, solid tumors implanted in nude mice as previously described:[3] MX-1 (breast), DLD-2 (colon), and LX-1 (lung). As before, DMP 840 displayed outstanding activity against all the tumor types. Table III also demonstrates that the four unsymmetrical compounds compared quite favorably to DMP 840. The compound showing the least amount of activity was *bis*-imide **10**. Two other compounds, **27** and **29**, were only tested in MX-1, but showed excellent in vivo activity with all tumors regressing. The most potent compound was DMP 315, which required about half the dose versus DMP 840 to obtain the same effect. DMP 315 displayed a broad spectrum of activity versus the three implanted tumors, as incomplete regressions (IR) were recorded for MX-1 and DLD-2. The LX-1 xenograft is our most stringent model, and DMP 315 showed excellent tumor growth inhibition (94%) at 6 mg/kg. In addition, DMP 315 displayed superior water solubility probably due in part to the unsymmetrical nature of the molecule. As a result of this activity and water solubility, DMP 315 has been advanced for further study.

Table III. In Vivo Results: Human, Solid Tumor Xenografts in Mice

Solid Tumor Name
% Tumor Growth Inhibition (MTD mg/kg, iv)
# FR and IR (full or incomplete regression)

Comp. #	MX-1	DLD-2	LX-1	H_2O Solubility
10	97% (12.5 mg/kg) 6 IR	98% (12.5 mg/kg) 3 IR	95% (10 mg/kg) 1 IR	29 g/L
27	- % (6.2 mg/kg) 9 IR	NT	NT	> 35.6 g/L
29	- % (6 mg/kg) 8 IR	NT	NT	NT
DMP 315	- % (6 mg/kg) 7 IR#	99% (6 mg/kg) 7 IR	94% (6 mg/kg)	65 g/L
DMP 840	>96% (10 mg/kg) 2 FR, 4 IR	99% (10 mg/kg) 1 FR, 6 IR	96% (10 mg/kg) 1 IR	3.5 g/L

In this experiment there were 7/8 survivors; NT = not tested
Description: Treatment (once daily X 9) with 8-10 mice began when the tumor weighed about 50 - 100mg, and the experiments were concluded 15-19 days after the initial treatment. The criteria of efficacy was the induction of regressed tumors (FR or IR) or percent of tumor growth inhibition (%TGI) relative to the control. When all FR or IR occur, the %TGI is not listed (- %).

Conclusions

We have introduced a new class of antitumor agent in the unsymmetrical *bis*-imides. These compounds demonstrate that the symmetry of DMP 840 is unnecessary for efficacy in vitro or in vivo. In general, the unsymmetrical compounds also display better water solubility. From these studies, we have identified DMP 315 as a promising candidate.

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