Nitration of Camptothecin with Various Inorganic Nitrate Salts in Concentrated Sulfuric Acid: A New Preparation of Anticancer Drug 9-Nitrocamptothecin

Zhisong Cao,* Kim Armstrong, Marcus Shaw, Eddie Petry, Nick Harris

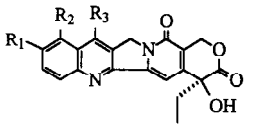
The Stehlin Foundation for Cancer Research and St. Joseph Hospital Cancer Laboratory, 1918 Chenevert Street, Houston, Texas 77003, USA
Tel. +1(713)7565750; Fax +1(713)7565783; E-mail: zcao@pipeline.com

Received 1 May 1998; revised 16 June 1998

Abstract: The nitration reactions of camptothecin **(1)** with 19 commonly used inorganic nitrate salts and a combination of two or more different nitrate salts in concentrated sulfuric acid are discussed. A new preparation of a promising anticancer drug 9-nitrocamptothecin **(4)** with a combination of potassium nitrate and thallium(I) nitrate as the nitrating reagents in concentrated sulfuric acid is described.

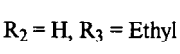
Key words: anticancer drugs, camptothecin, nitration, 9-nitrocamptothecin

Inhibitors of topoisomerase I, camptothecin **(1)**[1] and some of its semisynthetic derivatives such as topotecan **(2)**,[2] irinotecan (CPT-11) **(3)**,[3] and 9-nitrocamtothecin **(4)**,[4] have exhibited strong antitumor activity against various experimental tumor models including human lung, colon, and mammary tumor lines.[5–10] The water soluble compounds **2** and **3** are now commercially available as solutions and injectable for intravenous (i.v.) treatment. The water insoluble 9-nitrocamptothecin **(4)**, a nitration product of camptothecin, has been thoroughly studied both in vitro and in vivo in our laboratory,[11–20] demonstrating very potent antitumor activity against many different types of human cancers growing as xenografts in Swiss nude mice. Phase 1 human clinical trial with **4** was concluded by the Stehlin Foundation for Cancer Research. Phase 2 clinical trial with this drug against human pancreatic cancer is being conducted by the same institute and a very good response rate has been observed. Compound **4** has been approved by FDA to market as an orphan drug.



Camptothecin **1**: $R_1 = R_2 = R_3 = H$

Topotecan **2**: $R_1 = OH$, $R_2 = CH_2\text{-}N(CH_3)_2$, $R_3 = H$

Irinotecan **3**: R1 =  , $R_2 = H$, $R_3 = Ethyl$

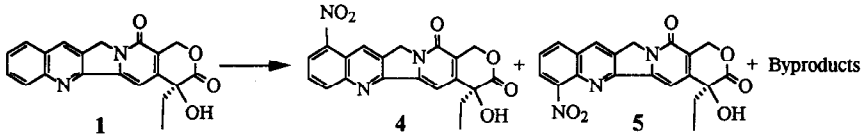
9-Nitrocamptothecin (9NC) **4**: $R_1 = R_3 = H$, $R_2 = NO_2$

Literature procedure for preparing compound **4** from camptothecin **(1)** is a conventional nitration reaction, which uses a nitric acid/sulfuric acid system as nitrating reagents.[4] When following this procedure to prepare **4** in our laboratory, a poor yield (from 3–7%) of the pure product **4** (good for patient-using) was obtained. This mixed acid procedure gave 9-nitrocamptothecin **(4)** and the inactive isomer 12-nitrocamptothecin **(5)** in a ratio of ca. 1:3 and about 20 other unidentified byproducts. Compound **5** was the major product (~60%). The unfavorable low ratio of **4** to **5** contributed to low yield of the active compound **4**. Too many other byproducts complicated the process of separation and purification, which decreased the yield of **4** as well. Thus, it is necessary to find an effective procedure to increase the yield of the anticancer compound **4** in order to meet the tremendous demand of clinical trials and laboratory uses (Scheme 1).

Inorganic nitrate salts have been used in the presence of strong mineral acids to nitrate organic compounds. Numerous examples may be found primarily in the patent literature. Olah reviewed the field of organic nitration reactions using a variety of inorganic nitrate salts as nitrating agents.[21] Except for strong inorganic acids, some organic acids and the corresponding anhydrides such as acetic acid, acetic anhydride, trifluoroacetic acid, and trifluoroacetic anhydride are also frequently employed as nitration reaction media.[22–25]

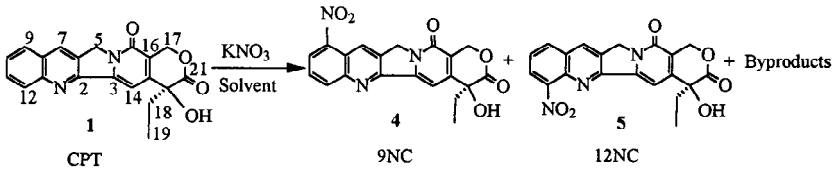
Employing inorganic nitrate salts as nitrating agents to nitrate anticancer agent camptothecin has not been reported before. In this paper we wish to report our results of the nitration of camptothecin with various inorganic nitrate salts in concentrated sulfuric acid.

In order to select a suitable reaction media for the nitration of camptothecin with various inorganic nitrate salts, the following solvents, acetic acid, acetic anhydride, trifluoroacetic anhydride, and concentrated sulfuric acid, were attempted while using potassium nitrate as nitrating agent. The results are shown in Table 1. When acetic acid and acetic anhydride were used as reaction medias no nitration reaction occurred. The starting camptothecin (CPT, 1) was recovered in 100% yield in both cases. In trifluoro-



Scheme 1

Table 1. Nitration of Camtothecin with KNO_3 in Different Solvents[a]



Solvent	Yield (%)				Ratio	
	9NC **4**	12NC **9**	CPT **1**	Byproducts	9NC/12NC	Total Nitration (%)
HOAc	0	0	100	0	0:0	0
Ac_2O	0	0	100	0	0:0	0
TFAA	4	8	23	65	1:2	12
H_2SO_4	21	47	26	6	1:2.2	68

[a] Camptothecin: (0.5 g), KNO_3 (0.5 g), solvent (30 mL), r.t., 24 h.

acetic anhydride, the nitration reaction happened, but the HPLC analyses of crude reaction mixture showed that the total nitration [9-nitrocamptothecin (%) + 12-nitrocamptothecin (%)] was only 12% in which only 4% was 9-nitrocamptothecin (9NC, **4**). The majority was unidentified byproducts. The HPLC analyses of unseparated reaction products for the nitration of camptothecin with KNO_3 in concentrated sulfuric acid showed a total nitration yield of 68% with a 9NC yield of 21%. The byproducts in this case were only 6%. Thus concentrated sulfuric acid was thoroughly employed as the media for all nitration reactions to be discussed.

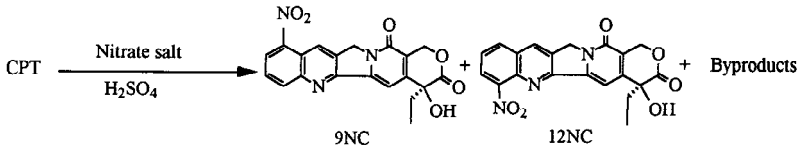
As shown in Table 1, nitration of camptothecin **(1)** with inorganic nitrate salt KNO_3 gave 9-nitrocamptothecin (9NC, **4**), 12-nitrocamptothecin (12NC, **5**), and many other byproducts. A good nitration reaction should be one that gives a higher percentage total nitration yield with a better ratio of percentage 9-nitrocamptothecin **(4)** to percentage 12-nitrocamptothecin **(5)**, and a lower percentage of byproducts.

The results of the nitration reaction of camptothecin with 19 commonly used inorganic nitrate salts in concentrated sulfuric acid are summarized in Table 2. All these nitrating reagents gave positive reactions, but the results were different. It is apparent that $BiONO_3$, $Cr(NO_3)$, and $La(NO_3)$ are not good nitrating agents under these reaction conditions. The 9NC values for these three salts were not good and were only 12 ± 1%. However, $TlNO_3$ showed good results: a better ratio of 9NC to 12NC (24%:49%, i.e., ~1:2.0), a higher total nitration value (73%), and a lower byproducts value (12%). KNO_3 also showed good results: the lowest byproducts value (6%), and the highest total nitration value (75%). But the ratio of 9NC to 12NC (23%:52%, i.e., 1:2.3) was not as good as with $TlNO_3$ as nitrating agent. Other salts such as $LiNO_3$, $Cu(NO_3)_2$, $Hg(NO_3)_2$, $Ca(NO_3)_2$, $Zn(NO_3)_2$, $Mg(NO_3)_2$, $Co(NO_3)_2$, $Sr(NO_3)_2$, and $Fe(NO_3)_3$ gave about the same results. All these 8 salts gave 9NC a value 20–23% and a good %total nitration value, but a higher %byproducts value was also observed for each of these eight salts, which increases difficulty for separation and purification. The remain salts in Table 2, NH_4NO_3, $AgNO_3$, $Ba(NO_3)_2$, $Pb(NO_3)_2$, and $Al(NO_3)_3$, belong to another category which gave 9-nitrocamptothecin **(4)** the values of 18 ± 1%. Thus, $TlNO_3$ and KNO_3, as nitrating agents, are better than others for the nitration of camptothecin under these reaction conditions.

The nitration of camptothecin with a combination of two different inorganic nitrate salts was attempted. The results are shown in Table 3. The comparison of Table 3 with Table 2 shows that some combination systems are better than when the two nitrates are used separately and some combinations are not as good as when they are used individually. For example, the combination of $LiNO_3$ and $Co(NO_3)_2$ gave a much lower 9NC value (11%), a lower total nitration value (41%), an unfavorable ratio of 9NC to 12NC (11%:30%, i.e., 1:2.7), and a much higher value of byproducts (57%). The following combination systems, $KNO_3/TlNO_3$, $KNO_3/Zn(NO_3)_2$, and $KNO_3/Sr(NO_3)_2$, showed improved results: higher %9NC values, more favorable ratios of %9NC to %12NC. The %total nitration values for these three systems are matchable with those in Table 2 with the corresponding individual salt as nitrating agent. The result obtained from $KNO_3/TlNO_3$ system is especially encouraging: a better ratio of %9NC to %12NC (1:1.4) and a higher 9NC value (29%) when compared with the values with KNO_3 or $TlNO_3$ as a nitrating agent separately. The $KNO_3/Hg(NO_3)_2$ system showed the best ratio of %9NC to %12NC (1:1.2), but the total nitration value was low (46%) and the value of byproducts was high (42%). Employment of a combination of three or more inorganic salts as the nitrating reagents was also attempted. The results are summarized in Table 4. All these combinations gave 9NC a 21–26% value. However, as shown in Table 3 the $KNO_3/TlNO_3$ combination is still the best one under our reaction conditions.

Thus, the $KNO_3/TlNO_3$ system was chosen to be studied further. Table 5 summarizes the results of the nitration of

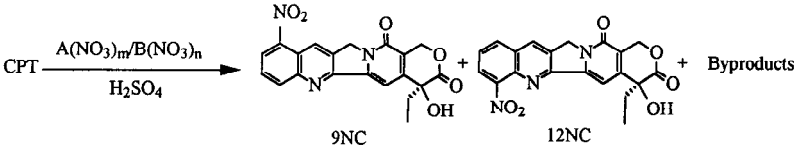
Table 2. Nitration of Camptothecin with Various Nitrates in Sulfuric Acid[a]



Nitrate Salt	Yield (%)				Ratio	
	9NC	12NC	CPT	Byproducts	9NC/12NC	Total Nitration (%)
NH_4NO_3	19	41	25	15	1:2.2	60
KNO_3	23	52	19	6	1:2.3	75
$LiNO_3$	22	38	0	40	1:1.7	60
$AgNO_3$	19	43	18	20	1:2.3	62
$TlNO_3$	24	49	15	12	1:2.0	73
$BiONO_3$	11	17	48	24	1:1.5	28
$Cu(NO_3)_2 \cdot 2.5\ H_2O$	22	53	1	24	1:2.4	75
$Hg(NO_3)_2 \cdot H_2O$	22	43	1	35	1:2.0	65
$Ca(NO_3)_2 \cdot 4\ H_2O$	20	49	1	30	1:2.5	69
$Ba(NO_3)_2$	17	54	3	20	1:3.2	71
$Zn(NO_3)_2 \cdot 6\ H_2O$	21	44	19	16	1:2.1	65
$Mg(NO_3)_2 \cdot 6\ H_2O$	23	45	9	23	1:2.0	68
$Co(NO_3)_2 \cdot 6\ H_2O$	21	41	0	38	1:2.0	62
$Sr(NO_3)_2$	22	36	1	41	1:1.6	58
$Pb(NO_3)_2$	18	50	13	19	1:2.8	68
$Al(NO_3)_3 \cdot 9\ H_2O$	17	46	1	36	1:2.7	63
$Fe(NO_3)_3 \cdot 9\ H_2O$	23	51	0	26	1:2.2	74
$Cr(NO_3)_3 \cdot 9\ H_2O$	13	28	46	13	1:2.2	41
$La(NO_3)_3 \cdot 6\ H_2O$	11	34	2	54	1:3.1	45

[a] For each reaction: Camptothecin (0.5 g, 0.0014 mol), H_2SO_4 (20 mL), nitrate (0.0070 mol), r.t., 72 h.

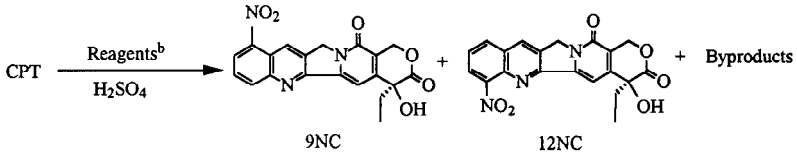
Table 3. Nitration of Camptothecin with a Combination of Two Different Nitrates in Sulfuric Acid[a]



$A(NO_3)_m/B(NO_3)_n$[b]	Yield (%)				Ratio	
	9NC	12NC	CPT	Byproducts	9NC/12NC	Total Nitration (%)
K/Cu[c]	24	50	6	20	1:2.1	74
K/Tl	29	41	8	22	1:1.4	70
K/Hg	21	25	12	42	1:1.2	46
K/Ca	18	42	3	37	1:2.3	60
K/Ba	20	39	0	41	1:2.0	59
K/Zn	26	50	10	14	1:1.9	56
K/Sr	29	45	3	23	1:1.6	74
K/Pb	24	49	12	15	1:2.0	73
K/Al	25	50	2	23	1:2.0	75
K/Fe	18	41	0	41	1:2.3	59
Li/Hg	17	38	17	26	1:2.2	55
Li/Cu	22	47	10	21	1:2.1	69
Li/Co	11	30	2	57	1:2.7	41
Ag/Cr	18	37	1	44	1:2.1	55
Cu/Fe	17	33	1	49	1:1.9	50
Hg/Fe	23	45	2	30	1:2.0	68
NH_4/Cu	17	49	2	32	1:2.9	66

[a] For each reaction: Camptothecin (4.0 g, 0.0115 mol), H_2SO_4 (100 mL), r.t., 72 h
[b] Molar ratio of $A(NO_3)_m$ to $B(NO_3)_n$: 2.0:1.0
[c] K/Cu represents the corresponding $KNO_3/Cu(NO_3)_2 \cdot 2.5\ H_2O$, same applied to the other combinations.

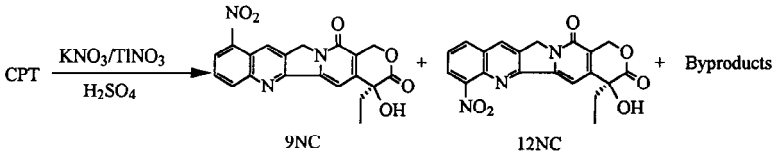
Table 4. Nitration of Camptothecin with a Combination of Three or More Nitrates in Sulfuric Acid[a]



Reagents	Yield (%)				Ratio	
	9NC	12NC	CPT	Byproducts	9NC/12NC	Total Nitration (%)
K/Tl/Cu[c]	25	44	10	21	1.0:1.8	69
K/Tl/Zn	25	46	0	29	1.0:1.8	71
K/Tl/Pb	25	46	5	24	1.0:1.8	71
K/Cu/Fe	22	48	6	24	1.0:2.2	70
K/Cu/Hg/Zn	24	57	2	17	1.0:2.4	81
K/Li/Cu/Hg/Fe	21	44	12	23	1.0:2.1	65
K/Li/Ag/Cu/Hg/Fe	24	50	4	22	1.0:2.1	74
K/Li/Zn/Cu/Hg/Fe	23	44	7	30	1.0:1.9	67
K/Zn/Cu/Hg/Fe	26	50	4	20	1.0:1.9	76

[a] For each reaction: Camptothecin (4.0 g, 0.0115 mol), H_2SO_4 (100 mL), r.t., 72 h.
[b] Molar ratio for all nitrates in a combination of the reagents is 1 to 1 to 1.
[c] K/Tl/Cu represents the corresponding $KNO_3/TlNO_3/Cu(NO_3)_2 \cdot 2.5\ H_2O$, same applied to other reagents.

Table 5. Nitration of Camptothecin with Different Ratios of a Combination of KNO_3 and $TlNO_3$ in Sulfuric Acid[a]

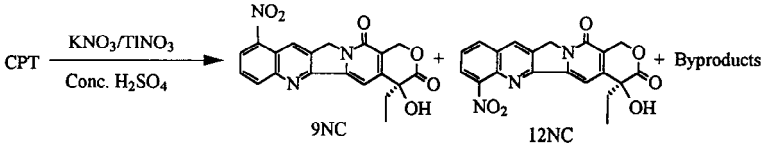


Ratio $KNO_3/TlNO_3$	Yield (%)				Ratio	
	9NC	12NC	CPT	Byproducts	9NC/12NC	Total Nitration (%)
3.3:1.0	26	47	5	22	1.0:1.8	73
2.0:1.0	29	41	8	22	1.0:1.4	70
1.4:1.0	27	46	10	17	1.0:1.7	73
1.3:1.0	28	49	6	17	1.0:1.8	77
1.2:1.0	27	50	8	15	1.0:1.9	77
1.0:1.0	26	49	11	14	1.0:1.9	75
1.0:1.3	27	49	11	13	1.0:1.8	76
1.0:1.5	26	47	12	15	1.0:1.8	73
1.0:2.1	25	38	9	28	1.0:1.5	63

[a] For each reaction: Camptothecin (6.0 g, 0.0172 mol), H_2SO_4 (100 mL), r.t. 72. h.

camptothecin with a combination of KNO_3 and $TlNO_3$ with different ratios in concentrated sulfuric acid. Once again, Table 5 shows that a higher %9NC value and a improved ratio of %9NC to %12NC are obtained when a combination of KNO_3 and $TlNO_3$ are used as the nitrating reagents. For example, when only KNO_3 was used as a nitrating agent the HPLC analysis of the reaction mixture showed that 9NC was 23% of the total area and 12NC was 52% of the same total area, the %9NC/%12NC ratio in this case was 1.0:2.3; when only $TlNO_3$ was used as a nitrating agent the same analysis showed a value of 24% for 9NC and 49% for 12NC. The ratio %9NC/%12NC in this case was 1.0:2.0. When these two nitrating reagents were used combined the 9NC value increased from 23.5 ± 0.5% to 27 ± 2% and the ratio of %9NC to %12NC increased from 1.0:2.15 ± 0.15 to 1.0:1.65 ± 0.25. The molar ratios of KNO_3 to $TlNO_3$ seem to have no influence on %total nitration, but it does have an effect on %byproducts. A higher %byproducts value makes the process of separation and purification more difficult. A combination with a ratio range of 1.0:1.0 to 1.0:1.5 of KNO_3 to $TlNO_3$ was chosen for the nitration reactions. The lowest %byproducts values were observed under these conditions. Generally, a combination with a ratio of 1.0 to 1.3 of KNO_3 to $TlNO_3$ is employed as the nitrating reagent in our laboratory for the preparation of 9-nitrocamptothecin **(4)**. A consistent yield of 20 ± 1% of pure **4** is obtained.

Table 6. Effects of the Volumes of Concentrated Sulfuric Acid on the Nitration of Camtothecin with a Combination of KNO_3 and $TlNO_3$[a]



H_2SO_4 (mL)	Yield (%)				Ratio	
	9NC	12NC	CPT	Byproducts	9NC/12NC	Total Nitration (%)
100	29	41	8	22	1.0:1.4	70
200	25	49	15	11	1.0:2.0	74
300	28	53	9	10	1.0:1.9	81
400	29	45	10	16	1.0:1.6	74

[a] Camptothecin (4.0 g), KNO_3 (2.0 g), $TlNO_3$ (4.0 g), r.t., 72 h.

Table 6 shows the effects of the volumes of concentrated sulfuric acid on the nitration of camptothecin with a combination of KNO_3 and $TlNO_3$. The better results are obtained when 300 mL of concentrated sulfuric acid is used as the reaction media while the amount of starting camptothecin is 4 grams.

Thus, the nitration procedure described in this report for the preparation of anticancer agent 9-nitrocamptothecin **(4)** from camptothecin **(1)** with a combination of two inorganic salts such as KNO_3 and $TlNO_3$ as nitrating reagents is superior to the conventional literature method (a mixed HNO_3/H_2SO_4 procedure[4]). The yield of pure 9-nitrocamptothecin **(4)** is improved from 5 ± 2% to 20 ± 1%. The concentrated sulfuric acid is recommended for the nitration reaction of camptothecin with all those inorganic salts discussed above.

Dry N_2 was routinely used as the reaction atmosphere in all reactions. All glassware was baked at 70 + 10 °C for a minimum of 2 h before used. Mps were obtained with a MEL–TEMP melting point apparatus and are uncorrected. The ^1H NMR spectra of approximately 10% (w/v) solution in $CDCl_3$ were obtained at 270.05 MHz with a JEOL GX-270 WB NMR spectrometer. Chemical shifts are reported in parts per million (δ scale), employing TMS as an internal standard. In reporting the NMR data, we have used the following abbreviations: coupling constants in Hz (*J*), singlet (s), doublet (d), triplet (t), broad singlet (bs), multiplet (m), and etc. MS were recorded using a VG ZAB–SEQ mass spectrometer (VG Analytical Co., England) with a resolution of 10000. Routinely used solvents such as $CHCl_3$ and CH_2Cl_2 were dried and freshly distilled. Silica gel (230–400 mesh, Aldrich) for column chromatography was used for all product separations. Eastman chromagram (silica gel with fluorescent indicator on polyethylene) sheets were employed in TLC operations. The numbering system used in reporting NMR data is shown in structure **1** of Table 1. Camptothecin was from The People's Republic of China and used as purchased. All other inorganic nitrate salts were from Aldrich Chemical Co. (Milwaukee, WI) and used as purchased.

Reaction of Camptothecin (1) with KNO_3 in Acetic Acid;Typical Procedure:
To HOAc (30 mL) in a 100-mL round-bottomed flask equipped with a magnetic stirrer were added camptothecin **(1)** (0.50 g, 0.0014 mol) and KNO_3 (0.50 g, 0.0050 mol). The mixture was stirred at r.t. for 24 h and poured onto ice-water (500 mL) portion-by-portion while stirring. The suspension was extracted with CH_2Cl_2 (3 × 200 mL). The combined extracts were dried (anhyd Na_2SO_4, 20 g, 6 h). After removal of CH_2Cl_2 by a rotary evaporator, the residue was refluxed in petroleum ether for 4 h. After filtration and drying in air for 4 h the product was obtained as gray white powders. HPLC analysis showed no nitration indicative of camptothecin with KNO_3 in acetic acid. The starting camptothecin was recovered 100%.

Reaction of Camptothecin (1) with KNO_3 in Acetic Anhydride:
Camptothecin **(1)** was nitrated and worked-up in the same way as the reaction in HOAc. The HPLC analysis of the reaction product showed 100% recovery of the starting camptothecin.

Reaction of Camptothecin (1) with KNO_3 in Trifluoroacetic Anhydride:
Camptothecin was nitrated and worked up in the same manner as in HOAc. The HPLC analysis data for the reaction mixture is shown in Table 1.

Nitration of Camptothecin (1) with KNO_3 in Concentrated Sulfuric Acid:
Camptothecin **(1)** (0.50 g, 0.0014 mol) and KNO_3 (0.50 g, 0.0050 mol) were added to concd H_2SO_4 (30 mL) in a 100-mL round-bottomed flask equipped with a magnetic stirrer all at once. The mixture was stirred at r.t. for 1 d and then poured onto ice-water (500 mL) slowly while stirring. The yellow suspension was extracted with CH_2Cl_2 (3 × 200). The combined extracts were dried (anhyd Na_2SO_4, 24 h). The CH_2Cl_2 was removed by a rotary evaporator and the residue was refluxed in petroleum ether for 4 h. After cooling to r.t. the mixture was filtrated and the yellow powders was dried in air for 1 d. The HPLC analysis data for the reaction mixture (yellow powder) is shown in Table 1.

Nitration of Camptothecin (1) with Various Nitrates in Sulfuric Acid; Typical Procedure:
Camptothecin **(1)** (0.50 g, 0.0014 mol) and NH_4NO_3 (0.56 g, 0.0070 mol) were added to concd H_2SO_4 (20 mL) in a 100-mL round-bottomed flask equipped with a magnetic stirrer. The mixture was stirred at r.t. for 72 h after which it was poured onto ice-water (500 mL) while stirring, extracted with CH_2Cl_2 (3 × 200 mL). The combined extracts were washed with water (2 × 100 mL). The two washes were combined and extracted with CH_2Cl_2 (2 × 100). All extracts (~600 mL + ~200 mL) were combined, dried (Na_2SO_4, 20 g, 4 h) and then evaporated to give crude reaction products as yellow powders which comprised of 9NC (19%), 12NC (41%), unreacted camptothecin **(1)** (25%), and many other byproducts (15%). The results of HPLC analyses for all these reactions are shown in Table 2.

Nitration of Camptothecin (1) with a Combination of Two Different Nitrates in Sulfuric Acid; Typical Procedure:
Camptothecin **(1)** (4.0 g, 0.0115 mol) was added to concd H_2SO_4 (100 mL) in a 250-mL three-necked flask. The suspension was stirred with a mechanical stirrer until the most of camptothecin went to solution (~15 to 30 min). To this solution KNO_3 (2.32 g, 0.0230 mol) and $Cu(NO_3)_2 \bullet 2.5\ H_2O$ (2.67 g, 0.0115 mol) were added all at once. The mixture was stirred at r.t. for 72 h and poured onto ice-water (1500 mL) while stirring. The yellow suspension in water was extracted with CH_2Cl_2 (4 × 500 mL). The combined extracts were dried (anhyd Na_2SO_4, 8 h). The Na_2SO_4 was removed by filtration. After removal of CH_2Cl_2 by a rotary evaporator, the crude reaction products were obtained as yellow powders, containing 9NC (24%), 12NC (50%), unreacted camptothecin (6%), and other byproducts (20%). The results of HPLC analyses for all these nitration reactions are shown in Table 3.

Nitration of Camptothecin (1) with a Combination of Three or More Inorganic Nitrates in Sulfuric Acid; Typical Procedure:
Camptothecin **(1)** (4.0 g, 0.0115 mol) was suspended in concd H_2SO_4 (100 mL) in a 250-mL round-bottomed flask equipped with a mechanical stirrer. After stirring for ~30 min (until camptothecin was almost dissolved), a combination of KNO_3 (1.16 g, 0.0115 mol), $TlNO_3$ (3.10 g, 0.0116 mol), and $Cu(NO_3)_2 \bullet 2.5\ H_2O$ (2.67 g, 0.0115 mol) was added all at once. The mixture was stirred at r.t. for 72 h and then poured onto ice-water (1500 mL) while stirring. The yellow suspension was extracted with CH_2Cl_2 (4 × 500 mL). The combined extracts were dried (anhyd Na_2SO_4), filtered, and evaporated. The crude reaction products were obtained as yellow powders, containing 9NC (25%), 12NC (44%), unreacted camptothecin (10%), and other byproducts (21%). The results of HPLC analyses for all these nitration reactions are shown in Table 4.

Nitration of Camptothecin (1) with Different Ratios of a Combination of KNO_3 and $TlNO_3$ in Sulfuric Acid; Typical Procedure:
Camptothecin **(1)** (6.0 g, 0.0172 mol) was added to concd H_2SO_4 (100 mL) in a 250-mL three-necked flask equipped with a mechanical stirrer. After stirring at r.t. for ~30 min, a combination of KNO_3 (1.74 g, 0.0172 mol) and $TlNO_3$ (4.58 g, 0.0172 mol) was added all at once. The mixture was stirred at r.t. for 72 h and then poured onto ice-water (1500 mL) while stirring. The yellow suspension was extracted with CH_2Cl_2 (4 × 500 mL). The combined extracts were dried (anhyd Na_2SO_4, 8 h). After removal of CH_2Cl_2 by a rotary evaporator, the crude reaction products were obtained as yellow powders, containing 9NC (26%), 12NC (49%), unreacted camptothecin (11%), and other byproducts (14%). The results of HPLC analyses for all these reaction are shown in Table 5.

Nitration of Camptothecin (1) with a Combination of KNO_3 and $TlNO_3$ in Various Volumes of Sulfuric Acid:
The general procedure was the same as above. The results was shown in Table 6.

9-Nitrocamptothecin (4):
Camptothecin **(1)** (4.0 g, 0.0115 mol) was added to concd H_2SO_4 (300 mL) in a 1000-mL three-necked flask. After stirring for ~15 min, KNO_3 (2.0 g, 0.0198 mol) and $TlNO_3$ (5.0 g, 0.0188 mol) were added all at once. The mixture was stirred at r.t. for 72 h and then poured onto ice-water (3500 mL) while stirring. The yellow suspension was extracted with CH_2Cl_2 (1 × 1500 mL and 2 × 900 mL). The combined extracts were dried (anhyd Na_2SO_4, 8 h). After filtration, the solvents were removed by a rotary evaporator. The residue was chromatographically separated. The crude 9-nitrocamptothecin was allowed to reflux in abs EtOH for 2–4 h. Pure product **4** was obtained by reprecipitation from EtOH to give bright yellow crystals; yield: 20%; mp 268 °C.

^{1}H NMR: δ = 1.05 (3H, t, J = 7.40 Hz, C19-methyl protons), 1.92 (2H, m, C18-methylene protons), 3.82 (1H, s, C20-OH), 5.40 (2H, s, C5-methylene protons), 5.55 (2H, dd, J = 14.21, 14.21 Hz, C17-methylene protons), 7.70 (1H, s, C14-H), 7.92 (1H, t, J = 8.40 Hz, C11-H), 8.48 (1H, d, J = 8.35 Hz, C10-H), 8.55 (1H, d, J = 8.35 Hz, C12-H), 9.36 (1H, s, C7-H).
MS: m/z (%) = 393 (M^+, 100), 364 ($M - C_2H_5$, 35), 349 (48), 334 (25), 320 (25), 293 (35), 274 (8), 262 (8), 246 (15), 234 (6), 218 (20), 205 (8), 190 (9), 177 (5), 164 (3), 151 (3), 137 (5), 123 (4), 109 (5), 95 (5), 75 (3), 60 (23).
HRMS: calcd for $C_{20}H_{15}N_3O_6$ 393.0960. Found 393.0961.

HPLC Procedure for Purity Analysis of 9-Nitrocamptothecin (4):
Instrumentation: The HPLC system consisted of a Beckman 421 controller with two 110A pumps and a 2 mL injection loop. The UV detector was a SPD-110AV model (Shimadzu, Kyoto, Japan). The HPLC detector was set to monitor the UV absorbance at 220 nm. The integrating software used for the analyses was EZChrome (Shimadzu, Japan) and FLO-ONE\beta (Radiomatic Instruments, Meridian, CT). A C-8 Microsorb was from Rainin Instruments (Woburn, MA).
HPLC analysis: Reverse phase HPLC analysis of the samples was carried out by using a MeCN/HOAc/H_2O mobile phase system. Analyses were carried out at r.t. with a flow rate 1 mL/min. The solution with a concentration of approximately 0.1 mg/mL of 9NC in MeCN was prepared by dissolving it in the solvent. A 300 µL portion of this solution was taken and added to 700 µL solution of 0.1% HOAc/H_2O. After shaking for ~10 s, 100 µL of this solution was injected through a 2 mL loop onto column and chromatographed with 70% H_2O with 0.1% HOAc and 30% MeCN as the mobile phase for the period of first 5 min, and then the gradient of the mobile phase was programmatically increased to 100% MeCN over a period of 4 min. A complete HPLC spectrum was obtained in 15 min. The purity of 9NC was determined by measuring the UV peak areas at 254 nm and calculating the percentage associated with the 9NC peak. The retention time of 9-nitrocamptothecin under these conditions is approximately 6.5 min.

HPLC Procedure for the Analyses of Crude Reaction Products:
The HPLC procedure for the analyses of crude nitration reaction products is the same as the one for the purity analysis of 9-nitrocamptothecin.

Financial support from the Stehlin Foundation for Cancer Research and The Friends of The Stehlin Foundation is gratefully acknowledged.

(1) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc*. **1966**, *88*, 3888.
(2) Kingsburg, W. D.; Boehm, L. C.; Jakas, D. R.; Holden, K. G.; Hecht, S. M.; Glagher, G.; Caranfa, M. J.; McCabe, F. L.; Faucette, L. F.; Johnson, R. K.; Hertzberg, R. P. *J. Med. Chem*. **1991**, *34*, 98.
(3) Sawada, S.; Matsuoka, S.; Nokata, K.; Nagata, H.; Furuta, T.; Yokokura, T., Miyasaka,T. *Chem. Pharm. Bull*. **1991**, *39*, 3183.
(4) Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem*. **1986**, *29*, 2358.
(5) Giovanella, B. C.; Stehlin, J. S.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Liu, L. F.; Silber, R.; Potmesil, M. *Sciences* (N.Y. Acad. Sci.) **1989**, *246*, 1046.
(6) Kawato, Y.; Furuta, T.; Aonuma, M.; Ysuoka, M.; Yokokura, T.; Matsumoto, K. *Cancer Chemother. Pharmacol*. **1991**, *28*, 192.
(7) Houghton, P. J.; Cheshire, P. J.; Myers, L.; Stewart, C. F.; Synold, T. W.; Houghton, J. A. *Cancer Chemother. Pharmacol*. **1992**, *31*, 229.

(8) Rowinsky, E. K.; Grochow, L. B.; Hendricks, C. B.; Ettinger, D. S.; Forastere, A. A.; Hurwitz, L. A.; McGuire, W. P.; Sartorius, S. E.; Lubejko, B. J.; Kaufman, S. H.; Donhower, R. C. *J. Clin. Oncol.* **1992**, *10*, 647.
(9) Negoro, S.; Fukuoka, M.; Masuda, N.; Takada, M.; Kusunoki, Y.; Matusi, K.; Takifuji, N.; Kudoh, S.; Niitani, H.; Taguchi, T. *J. Natl. Cancer Inst.* **1991**, *83*, 1164.
(10) Fukuoka, M.; Niitani, H.; Suzuki, A.; Motomiya, M.; Haesgawa, K.; Nishiwaki, Y.; Kuriyama, T.; Airyoshi, Y.; Negoro, S.; Masuda, N.; Nakajima, S.; Taguchi, T. *J. Clin. Oncol.* **1992**, *10*, 16.
(11) Pantazis, P.; Early, J. A.; Kozielski, A. J.; Mendoza, J.; Hinz, H.; Giovanella, B. *Cancer Res.* **1993**, *53*, 1577.
(12) Pantazis, P.; Mendoza, J.; Early, J.; Kozielski, A.; Natelson, E.; Giovanella, B. *Eur. J. Haematol* **1993**, *50*, 81.
(13) Hinz, H.; Harris, N.; Natelson, E.; Giovanella, B. *Cancer Res.* **1994**, *54*, 3096.
(14) Pantazis, P.; Hinz, H.; Mendoza, J.; Kozielski, A.; Williams, L.; Stehlin, J.; Giovanella, B. *Cancer Res.* **1992**, *52*, 3980.
(15) Pantazis, P.; Kozielski, A.; Vardeman, D.; Petry, E.; Giovanella, B. *Oncology Res.* ***1993***, *5*, 273.
(16) Pantazis, P.; Harris, N.; Mendoza, J.; Giovanella, B. *Eur. J. Haematol.* **1994**, *53*, 246.
(17) Pantazis, P.; Mendoza, J.; DeJesus, A.; Rubin, E.; Kufe, D.; Giovanella, B. *Eur. J. Haematol.* **1994**, *53*, 135.
(18) Pantazis, P.; Early, J.; Mendoza, J.; DeJesus, A.; Giovanella, B. *Cancer Res.* **1994**, *54*, 771.
(19) Rubin, E.; Pantazis, P.; Bharti, A.; Toppmeyer, D.; Giovanella, B.; Kufe, D. *J. Biol. Chem.* **1994**, *269*, 2433.
(20) Pantazis, P.; Kozielski, A.; Mendoza, J.; Early, J.; Hinz, H.; Giovanella, B. *Eur. J. Haematol.* **1993**, *53*, 863.
(21) Olah, G. A. *Aldrichchimica Acta* **1979**, *12*, 43.
(22) Tedder, J. M. *Chem. Rev.* **1955**, *55*, 287.
(23) Bourne, E. J.; Stacey, M.; Totlow, J. C.; Tedder, J. M. *J. Chem. Soc.* **1952**, 1695.
(24) Stock, L. M.; Wright, T. L. *J. Org. Chem.* **1979**, *44*, 3467.
(25) Crivello, J. V. *J. Org. Chem.* **1981**, *46*, 3056.