Synthesis, Tubulin Binding, Antineoplastic Evaluation, and Structure-Activity Relationship of Oncodazole Analogues

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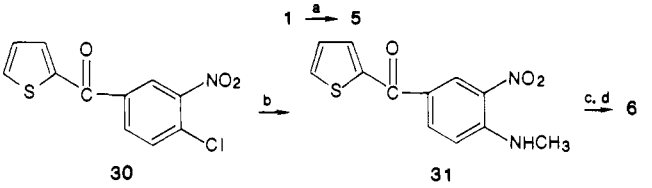
In an attempt to identify a soluble oncodazole analogue that could be easily formulated, a series of substituted oncodazoles was synthesized and evaluated for tubulin binding affinity, in vitro cytotoxicity against cultured mouse B-16 cells, and ability to prolong lifespan at the maximally tolerated dose in the P388 mouse leukemia model. Biological evaluation of all the isomeric methyloncodazoles demonstrated the thiophene 4′-position to be the only site of significant bulk tolerance, although substitution of this position with polar or charged functional groups abolished biological activity. Simple esters of the 4′-carboxymethyloncodazole were shown to have enhanced antitumor activity and tubulin binding affinity relative to oncodazole. Despite a failure of this study to identify a water-soluble oncodazole with antitumor activity, the structure-activity relationship developed led to a derivative with enhanced activity in the P388 leukemia model and facilitated the preparation of a biologically active photolabile analogue.

Oncodazole, 1, methyl [5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate, shows antifungal,[1] antineoplastic,[2] and anthelmintic[3] activities which are generally believed to result from binding of 1 to tubulin and consequent disruption of the mitotic spindle.[4] The high crystallinity and insolubility of 1 lead to poor and irreproducible oral bioavailability particularly as an antitumor agent; this, together with the relatively modest activity of 1 in animal tumor models, has precluded development of this compound as an antineoplastic. Compound 1 therefore became an interesting lead structure for synthetic modification with the aims of increasing solubility, ease of formulation, and antitumor activity. An additional goal was the preparation of a photolabile analogue of 1 which made use of a developing structure–activity relationship (SAR) to retain tubulin binding affinity and cytotoxic activity while yielding an irreversible tubulin binding agent as a tool for protein chemistry efforts. Here we report a systematic study of some simple alkyl analogues of 1 which yielded information about sites of bulk tolerance in the tubulin site which binds 1. This study has resulted in several analogues of 1 which show increased in vitro binding affinity for tubulin and improved antineoplastic activity in the P388 mouse leukemia model. Several more soluble analogues of 1 were also prepared, but these were found to be significantly less active than the parent molecule. An application of the SAR developed as part of this study has already led to the discovery of a photolabile analogue of 1 which retains significant affinity for tubulin.[5]

Chemistry

Synthesis of the compounds in Table I was carried out by the general methodology outlined in Schemes I–XI. It was found during the course of this work that the *p*-toluenesulfonic acid catalyzed reaction of ortho diamines with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea in MeOH is the method of choice for forming the required benzimidazoles.[6] Compounds 1–4 were prepared by the literature method,[3] and compound 5 was prepared by methylating a DMF solution of the sodium salt of oncodazole (Scheme I). The regiochemical outcome of this alkylation, which gave predominantly a single isomer, was determined by comparison of 5 with the other two possible methyl isomers, 6 and 7, which were prepared by unambiguous routes.

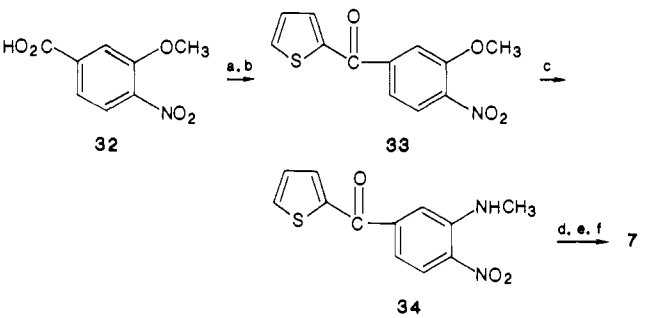
Scheme I[a]



30 31

[a] Reagents and conditions: (a) NaH, CH_3I, DMF; (b) CH_3NH_2, DMSO; (c) H_2, Pd/C, MeOH, 65 °C; (d) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, dioxane, H_2O.

Scheme II[a]



32 33 34

[a] Reagents and conditions: (a) $SOCl_2$, trace DMF, $CHCl_3$; (b) $AlCl_3$, C_4H_4S, CH_2Cl_2; (c) CH_3NH_2, DMSO, 85 °C; (d) H_2, Pd/C, MeOH; (e) HCl; (f) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, H_2O.

Synthesis of 1-methyloncodazole (6) was accomplished as shown in Scheme I. Nucleophilic displacement of the chloro nitro ketone 30[3] with methylamine yielded nitroaniline 31, which was reduced and cyclized to 6. The isomeric N3-methyl compound 7 was prepared in a similar way (Scheme II).

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(1) Davidse, L. C.; Flach, W. *Biochim. Biophys. Acta* **1978**, *543*, 82.
(2) Lacey, E.; Watson, T. R. *Biochem. Pharmacol.* **1985**, *34*, 3603 and references cited therein.
(3) Raeymaekers, H. H. M.; Van Gelder, J. L. H.; Roevens, L. F. C.; Janssen, P. A. J. *Arzneim.-Forsch./Drug Res.* **1978**, *28*, 586.
(4) (a) De Brabander, M.; Van de Veire, R.; Aerts, F.; Geuens, G.; Brogers, M.; Desplenter, L.; De Cree, J. In *Microtubules and Microtubule Inhibitors*; Borgers, M., De Brabander, M., Eds.; Elsevier: New York, 1975; pp 509–521. (b) De Brabander, M. J.; Van de Veire, R. M. L.; Aerts, F.; Borgers, M.; Janssen, P. A. J. *Cancer Res.* **1976**, *36*, 1011.
(5) Ladd, D. L.; Harrsch, P. B.; Kruse, L. I. *J. Org. Chem.* **1988**, *53*, 417.
(6) Rajappa, S.; Sreenivasan, R. *Indian J. Chem.* **1985**, *24B*, 14.

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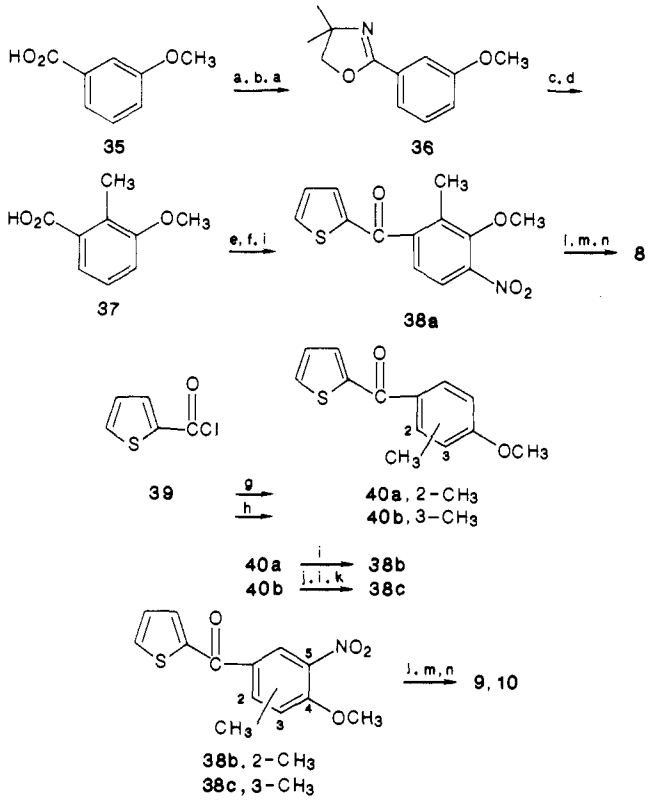
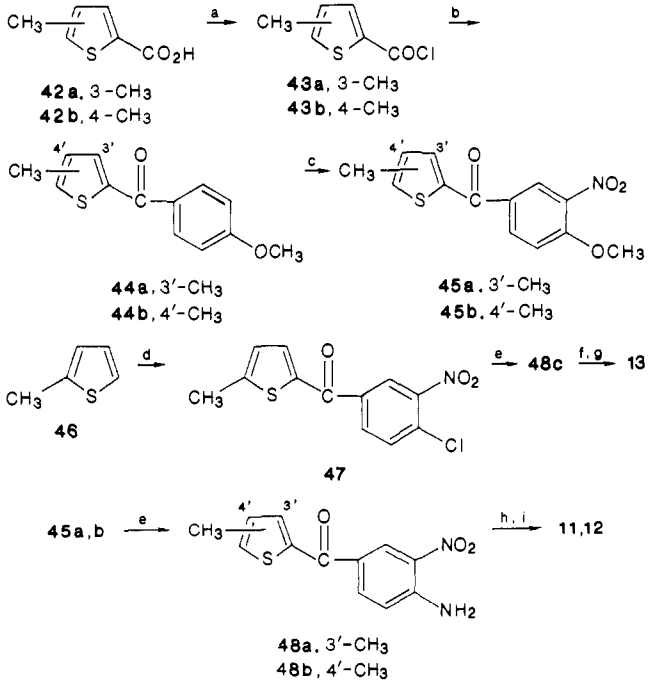
Scheme III[a]



[a] Reagents and conditions: (a) $SOCl_2$; (b) $NH_2C(CH_3)_2CH_2OH$; (c) *n*-BuLi, then CH_3I; (d) HCl; (e) $SOCl_2$, $CHCl_3$; (f) $AlCl_3$, C_4H_4S; (g) $AlCl_3$, *m*-$(CH_3O)C_6H_4CH_3$, CH_2Cl_2; (h) $AlCl_3$, *o*-$(CH_3O)C_6H_4CH_3$, CH_2Cl_2; (i) HNO_3, H_2SO_4, CH_2Cl_2; (j) BBr_3, CH_2Cl_2; (k) $(CH_3O)_2SO_2$, K_2CO_3, DMF; (l) NH_3, DMSO; (m) H_2, Pd/C, MeOH; (n) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, MeOH.

Scheme IV[a]

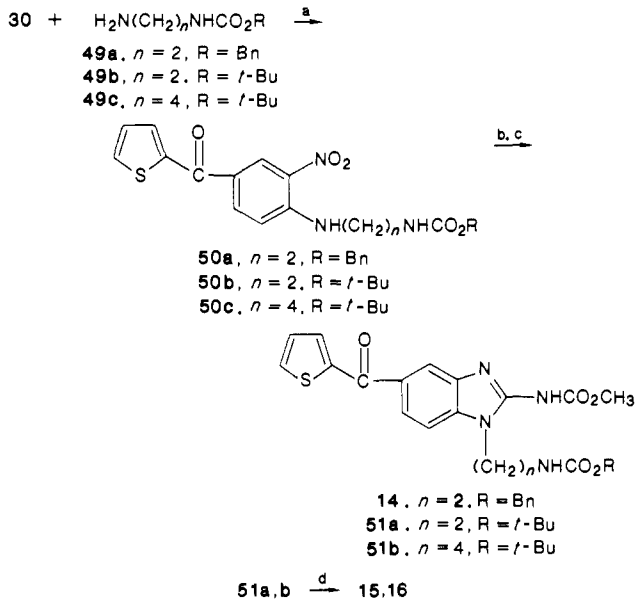


[a] Reagents and conditions: (a) $SOCl_2$, $CHCl_3$; (b) $AlCl_3$, C_6H_5O-CH_3, CH_2Cl_2; (c) HNO_3, Ac_2O; (d) $AlCl_3$, 3-(NO_2)-4-Cl-C_6H_3COCl, CH_2Cl_2; (e) NH_3, DMSO; (f) H_2, Pd/C, MeOH, then HCl; (g) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, H_2O; (h) H_2, Pd/C, MeOH; (i) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, CH_3OH.

Synthesis of the three isomeric benzimidazoles methylated in the phenyl ring, **8–10**, was conducted via the strategy in Scheme III. The anisic acid intermediate **37** was prepared by lithiation and methylation of the oxazoline **36**[7] followed by hydrolysis. Friedel–Crafts acylation of thiophene with the acid chloride prepared from **37** followed by nitration gave **38a**. Acylating the isomeric 2- and 3-methylanisoles with thiophene-2-carbonyl chloride followed by nitration yielded methoxy nitro ketones **38b,c**. In the case of **40b**, it was found necessary to nitrate the corresponding phenol to provide acceptable yields of the required regioisomer. Nucleophilic displacement of the methoxyl groups in **38a–c** followed by hydrogenation and cyclization yielded benzimidazoles **8–10**.

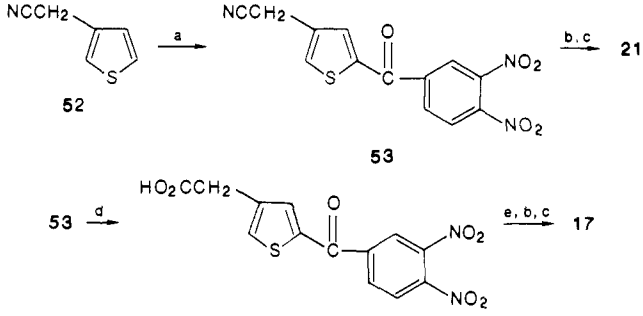
The three methylthiophene isomers **11–13** were prepared via related routes as outlined in Scheme IV. The isomeric methoxy nitro ketone intermediates **45a,b** were prepared by Friedel–Crafts acylation of anisole with 3- and 4-methylthiophenecarbonyl chloride followed by nitration. Ketone **47**, an intermediate to the remaining 5'-methylthiophene isomer was prepared in a slightly different way by acylating 2-methylthiophene with 4-chloro-3-nitrobenzoyl chloride. Nucleophilic displacement of the methoxyl groups from **45a,b** and the chlorine from **47** by ammonia yielded the nitroanilines **48a–c**, which upon re-

Scheme V[a]



[a] Reagents and conditions: (a) NEt_3, DMSO; (b) H_2, Pd/C or Pt_2O, MeOH; (c) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, CH_3OH; (d) HCO_2H.

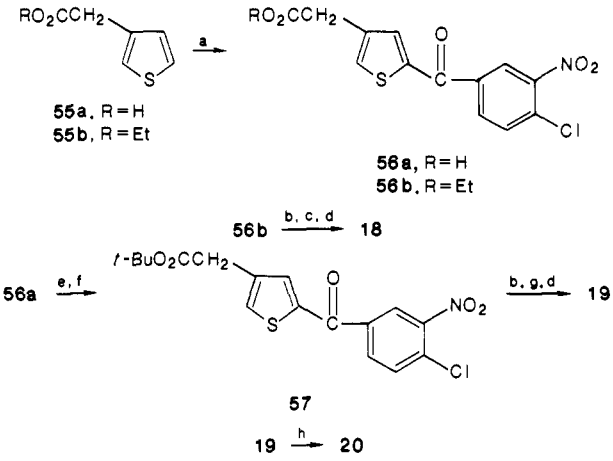
Scheme VI[a]



[a] Reagents and conditions: (a) $AlCl_3$, 3,4-$(NO_2)_2$-C_6H_3COCl, CH_2Cl_2; (b) H_2, Pd/C, MeOH; (c) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, CH_3OH; (d) H_2SO_4; (e) CH_3OH, HCl.

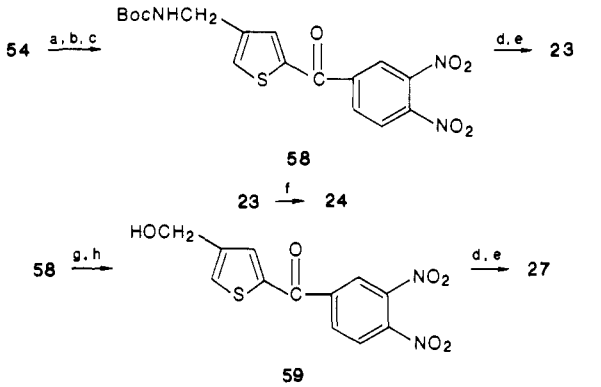
(7) Meyers, A. I.; Avila, W. B. *J. Org. Chem.* 1981, *46*, 3881.

Scheme VII[a]



[a] Reagents and conditions: (a) $AlCl_3$, 3-(NO_2)-4-Cl-C_6H_3COCl, CH_2Cl_2; (b) NH_3, DMSO; (c) H_2, Pd/C, EtOH; (d) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, CH_3OH; (e) $SOCl_2$, CH_2Cl_2; (f) $(CH_3)_3C$-OH; (g) H_2, Pd/C, MeOH; (h) HCO_2H.

Scheme VIII[a]



[a] Reagents and conditions: (a) $SOCl_2$; (b) $TMSN_3$, $C_6H_5CH_3$; (c) *t*-BuOH; (d) H_2, Pd/C, MeOH; (e) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, CH_3OH; (f) HCO_2H; (g) HCl, EtOAc; (h) $NaNO_2$, TsOH, DMSO, H_2O.

duction and cyclization yielded **11–13**.

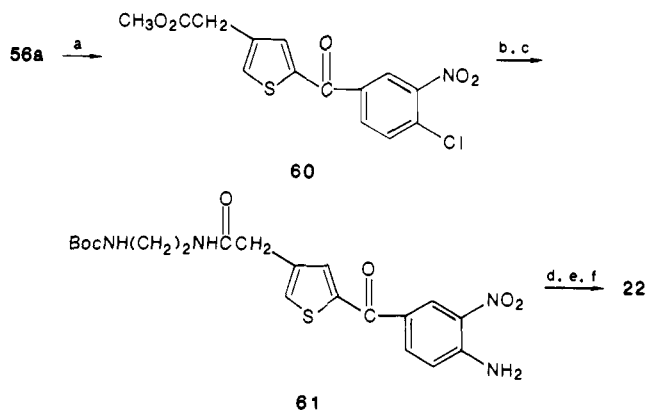
The nucleophilic displacement of chloro nitro ketone **30** with half-protected 1,2-diaminoethanes **49a,b** or 1,4-diaminobutane **49c** provided nitroanilines **50a–c**, which upon reduction, cyclization, and deprotection (when appropriate) yielded **14–16** (Scheme V).

As shown in Scheme VI, acylation of 3-(cyanomethyl)-thiophene (**52**) with 3,4-dinitrobenzoyl chloride followed by reduction and cyclization gave **21**. Alternatively, acid-catalyzed hydrolysis of the intermediate nitrile **53** followed by esterification, reduction, and cyclization provided ester **17**. In a related strategy (Scheme VII) acylation of thiophene acid **55a** or thiophene ester **55b** yielded intermediate ketones **56a,b**. Displacement of **56b** with ammonia followed by reduction and cyclization yielded ethyl ester **18** whereas esterification of **56a** followed by a similar sequence gave *tert*-butyl ester **19** or, upon deprotection of **19** with formic acid, the acid **20**.

The 4′-(hydroxymethyl)- and 4′-(aminomethyl)-thiophenes (**27** and **24**, respectively) were prepared from acid **54** as shown in Scheme VIII. Modified Curtius reaction[8] of the acid chloride prepared from **54** gave the intermediate **58**, which upon reduction and cyclization

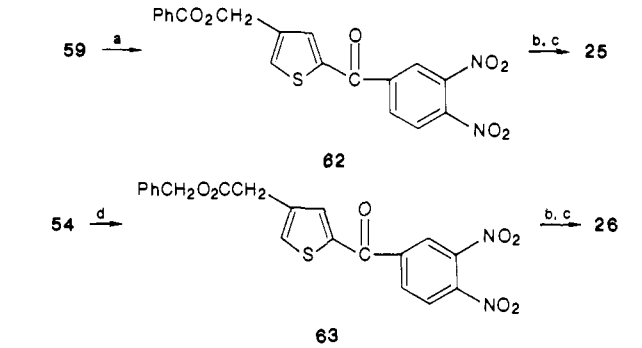
(8) (a) Washburne, S. S.; Peterson, W. R., Jr. *Synth. Commun.* **1972**, *2*, 227. (b) MacMillan, J. H.; Washburne, S. S. *J. Org. Chem.* **1973**, *38*, 2982.

Scheme IX[a]



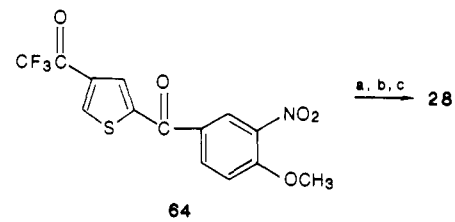
[a] Reagents and conditions: (a) CH_3OH, HCl; (b) NH_3, DMSO; (c) $NH_2(CH_2)_2NHBoc$, 2-OH-C_5H_4N, 120 °C; (d) H_2, Pd/C, MeOH; (e) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, CH_3OH; (f) HCO_2H.

Scheme X[a]



[a] Reagents and conditions: (a) $PhCO_2H$, 4-pyrrolidinopyridine, DCC, dioxane, CH_2Cl_2; (b) $Na_2S_2O_4$, THF, H_2O; (c) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, MeOH; (d) $PhCH_2OH$, 4-pyrrolidinopyridine, DCC, dioxane, CH_2Cl_2.

Scheme XI[a]



[a] Reagents and conditions: (a) NH_3, DMSO, 85 °C; (b) H_2, Pd/C, MeOH; (c) $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, TsOH, MeOH.

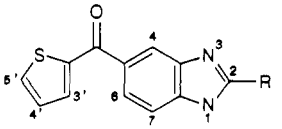
yielded **23**. Acid-catalyzed deprotection of **23** provided the (aminomethyl)thiophene **24**. Deprotection of carbamate **58** followed by diazotization and in situ solvolysis yielded the 4′-hydroxymethyl intermediate **59**, which upon reduction and cyclization afforded benzimidazole **27**.

Scheme IX outlines the preparation of *N*-(aminoethyl)carboxamide **22** from ester **60**. Displacement of the aryl chloride **60** with ammonia, followed by reaction of the resulting ester with **49b**, provided intermediate **61**. Reduction, cyclization, and deprotection yielded benzimidazole **22**.

Synthesis of the ester **25** and its "reversed ester" counterpart **26** was accomplished as outlined in Scheme X. The DCC-mediated couplings of **59** with benzoic acid and **54** with benzyl alcohol yielded ester intermediates **62** and **63**, respectively. Chemical reduction of **62** and **63** followed by cyclization yielded **25** and **26**.

The 4′-[(trifluoromethyl)carbonyl]thiophene analogue **28** was prepared from the previously reported[5] interme-

Table I. Structure, Physical Properties, and Biological Activity of Some Substituted Oncodazoles



no.	R	substit	mp, °C	tubulin binding,[a] μM	cytotoxicity: IC_{50},[b] μM	in vivo[c] MTD,[d] mg/kg	in vivo[c] activity,[e] % ILS
1	$NHCO_2CH_3$			5.7 ± 1.9	0.1	80	75 ± 23[f]
2	$NHCO_2CH_2CH_3$				0.2	160	neg[g]
3	$NHCO_2CH_2CH_2CH_3$				0.5	>320	neg
4	$NHCO_2CH(CH_3)_2$			14.0 ± 3.0	0.2	320	45, 30[h]
5	$N(CH_3)CO_2CH_3$		171–172.5		50	40	neg
6	$NHCO_2CH_3$	1-CH_3	189–196		20	160	neg
7	$NHCO_2CH_3$	3-CH_3	190–193		40	>320	neg
8	$NHCO_2CH_3$	4-CH_3	296–301 dec	106 ± 46	4	40	neg
9	$NHCO_2CH_3$	6-CH_3	248–255 dec		8	>320	neg
10	$NHCO_2CH_3$	7-CH_3	300–306 dec	0.86 ± 0.23	0.06	30	45, 50[h]
11	$NHCO_2CH_3$	3′-CH_3	235–243 dec		2	>320	neg
12	$NHCO_2CH_3$	4′-CH_3	294–297 dec	17.6 ± 2.0	0.1	80	95 ± 23[f,i]
13	$NHCO_2CH_3$	5′-CH_3	297–301 dec		10	160	neg
14	$NHCO_2CH_3$	1-$(CH_2)_2NHCBZ$	181–184		20		
15	$NHCO_2CH_3$	1-$(CH_2)_2NH_2$	142 dec		30	80	neg
16	$NHCO_2CH_3$	1-$(CH_2)_4NH_2$	141 dec		30	80	neg
17[j]	$NHCO_2CH_3$	4′-$CH_2CO_2CH_3$	220–222 dec	5.0 ± 1.0	0.04	30	65
18	$NHCO_2CH_3$	4′-$CH_2CO_2CH_2CH_3$	239–241 dec	0.88 ± 0.26	0.02	80	55, 55[h]
19	$NHCO_2CH_3$	4′-$CH_2CO_2C(CH_3)_3$	195.5–199 dec	7.5 ± 0.8	0.07	160	105, 50[h]
20[j]	$NHCO_2CH_3$	4′-CH_2CO_2H	244 dec	442 ± 59	2	80	neg
21	$NHCO_2CH_3$	4′-CH_2CN	286 dec	2.9 ± 0.3	0.02	160	80, 80[h]
22[j]	$NHCO_2CH_3$	4′-$CH_2CONH(CH_2)_2NH_2$	172 dec		>70	80	neg
23	$NHCO_2CH_3$	4′-CH_2NHBOC	219 dec	23.3 ± 3.0	0.5	160	neg
24	$NHCO_2CH_3$	4′-CH_2NH_2	amorphous	120 ± 20	2	20	neg
25	$NHCO_2CH_3$	4′-CH_2OCOPh	223.5–228	1.95 ± 0.7			
26	$NHCO_2CH_3$	4′-CH_2CO_2Bn	194–196 dec	5.2 ± 1.0			
27	$NHCO_2CH_3$	4′-CH_2OH	275.5 dec	22 ± 9	1.8		
28	$NHCO_2CH_3$	4′-$COCF_3$	277 dec	16 ± 5	0.14		
29[j]	$NHCO_2CH_3$	4′-$C(N_2)CF_3$	>400 dec	8.3 ± 3	0.14		

[a] IC_{50} values in μM (mean ± SEM) for concentration needed to displace 5.8 μM [^{3}H]oncodazole from soluble, homogeneous tubulin. [b] Concentration which inhibited proliferation of monolayer B16 melanoma cells by 50% on continuous 72-h exposure.[9a] [c] Data obtained in syngenic B6D2F_1 female mice bearing ip implanted (10^6 cells) P388 leukemia. Compounds were compared directly with 1 in all studies. [d] Maximally tolerated dose in mg/kg per day; drugs were administered ip on days 1–5 following tumor implantation at dose levels of 320–20 mg/kg per day. [e] Increase in lifespan at maximally tolerated dose based on median survival time relative to untreated controls. [f] Values are mean ± SD for multiple dose–response studies. [g] A compound is considered negative if it produced less than 25% increase in lifespan at the maximally tolerated dose.[9b] [h] Two values obtained in independent dose–response studies. [i] The activity of 12 was significantly greater ($p < 0.05$) than that of 1 in a paired Student's t test. [j] Despite apparent purity by NMR and HPLC, satisfactory CHN analysis could not be obtained.

diate **64** by ammonia displacement, hydrogenation, and cyclization (Scheme XI).

Biology

The benzimidazoles in Table I were evaluated in several biological assays in an attempt to correlate in vitro cytotoxicity with tubulin binding and antitumor efficacy in the P388 mouse leukemia model.

Tubulin binding was determined under equilibrium conditions by the displacement of ^{3}H-labeled oncodazole from homogeneous tubulin as previously reported.[5] This equilibrium binding assay allows the reliable determination of tubulin binding affinity even for those analogues which bind very weakly.

In vitro cytotoxicity was determined for cultured mouse B-16 melanoma cells as previously described.[9a]

The in vivo efficacy of selected compounds was measured at the maximally tolerated dose in the P388 mouse leukemia model, according to the earlier experimental protocol.[9b]

Results and Discussion

The goal of this study was to define the in vitro tubulin binding affinity and in vivo antineoplastic activity of a series of substituted oncodazoles with the objective of developing a water-soluble analogue that could be formulated easily and a photolabile analogue which retained full biological activity. In light of these objectives, the present study was only partially successful.

Initial investigation consisted of a systematic evaluation of all the possible monomethyloncodazole isomers to determine bulk tolerance in a series of molecules which deviated little from the overall physicochemical properties of the parent, oncodazole (**1**). Data (Table I) from this series of compounds (**2**, **5–13**) was remarkable in that it showed a complete divergence between cytotoxicity, % ILS in P388, and, where measured, tubulin binding affinity. Two compounds, **10** and **12**, provide extreme examples of this. The 7-methyl analogue, **10**, which has greatly enhanced tubulin binding affinity relative to **1**, is more cytotoxic and is less well tolerated in mice, but is of diminished efficacy in the P388 model. Compound **12**, the 4′-methyl analogue of **1**, which has significantly reduced tu-

(9) (a) Mirabelli, C. K.; Bartus, H.; Bartus, J. O'L.; Johnson, R.; Mong, S. M.; Sung, C. P.; Crooke, S. T. *J. Antibiotic* **1985**, *38*, 758. (b) Mirabelli, C. K.; Johnson, R. K.; Hill, D. T.; Faucette, L. F.; Girard, G. R.; Kuo, G. Y.; Sung, C. M.; Crooke, S. T. *J. Med. Chem.* **1986**, *29*, 218.

bulin binding relative to 1, is significantly more active in vivo in the P388 model.

Compound 4, which has activity in the P388 model, is a 2-propyl carbamate. This compound was less efficacious than 1. Some simple 2-propyl *N*-arylcarbamates have been reported to block mitosis through a poorly defined action on tubulin or the microtubule organizing centers,[10] leading to the possibility that the activity of 4 may result from several modes of action. All of the other methyloncodazole isomers were without significant activity in vivo. The three additional N1-substituted compounds 14–16 were without significant activity.

Once the activity of the 4′-methyloncodazole 12 had been established, further modification at this site was carried out in an attempt to prepare a water-soluble analogue. Compounds with charged, solubilizing 4′-side chains were prepared, but these were found to be totally devoid of activity. The inactivity of compounds with negatively (20) or positively charged (22, 24) side chains clearly resulted from the charge and not the increased bulk of these substituents, as evidenced by comparing activities of the appropriate compounds in Table I. Thus, acid 20 was without significant activity, whereas the corresponding esters, methyl (17), ethyl (18), and even *tert*-butyl (19), were active both in vitro and in vivo.

The SAR resulting from changes at the thiophene ring of oncodazole identify this portion of the molecule as a critical region for interaction with the β-tubulin binding site. It is interesting to note that the 4′-methyl analogue 12 binds β-tubulin 2–3-fold less tightly than 1 whereas in vitro cytotoxicity and in vivo MTD are identical. In the mouse P388 model of leukemia, 12 is significantly more active than 1. Indeed, this compound is the most potent oncodazole analogue identified to date. In terms of simple binding to β-tubulin, those analogues with small but polar uncharged groups at the 4′-position appear to be most effective. Thus, esters 18 and 25 are very potent binding agents, as is the cyanomethyl analogue 21. The polar (but possibly hydrated) trifluoromethyl ketone 28 is of diminished binding potency relative to 1. As noted above, imposition of either positive or negative charge at this position abolishes activity.

It is instructive to consider the more general issue of whether in vitro binding affinity for β-tubulin is a predictor of antineoplastic activity in the mouse P388 model. A comparison of β-tubulin binding data vs in vitro cytotoxicity for those compounds (n = 15) in Table I where both values are available show a very good correlation (r^2 = 0.77). However, a direct correspondence between cytotoxicity and in vivo activity in the P388 model is clearly not shown by the data in Table I. Tubulin binding of oncodazole and its analogues appears not to correlate with antineoplastic efficacy, although all of those compounds which show in vivo activity in the P388 model *are* potent β-tubulin binding agents. Several possibilities could explain the divergence between in vitro β-tubulin binding and in vivo antineoplastic activity. Firstly, a selective uptake, distribution, or metabolism of the β-tubulin binding agent may be important. Secondly, β-tubulin binding potency, as measured here, will reflect affinity for the heterogeneous mix of β-tubulin isoforms (β_1, β_2, β_n) that have been shown to occur in vivo.[11] Effective antitumor activity may result from a selective binding to a single β-tubulin isoform.

In summary, a simple series of oncodazole analogues has been prepared and evaluated systematically to determine effects on β-tubulin binding, cytotoxicity, and antineoplastic activity in the mouse P388 model. This study identified an oncodazole analogue with improved in vivo activity. A correlation between in vitro cytotoxicity and β-tubulin binding was demonstrated for a number of analogues.

Experimental Section

Melting points are uncorrected. Elemental analyses, high-field ^{1}H NMR, and mass spectra were obtained by the Analytical Department of Smith Kline & French Laboratories. Mass spectra were obtained on Varian-MAT CH-5 DF (DCI) and VG ZAB-1F (FAB) spectrometers; ^{1}H NMR spectra were obtained with Varian EM-390, JEOL JNM-GX270, Bruker WP 360, and AM 250 spectrometers. The identity of all new compounds was confirmed by both mass spectral and NMR data; homogeneity was confirmed by either HPLC or TLC. Catalytic hydrogenations were carried out in a Parr apparatus at an initial pressure of 50 psi. Solutions were routinely dried over anhydrous magnesium sulfate prior to evaporation. Flash chromatography was carried out according to Still.[12]

***N*-(Methoxycarbonyl)-*N*-methyl-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-amine (5).** A suspension of 1 (1.5 g, 4.98 mmol) in 150 mL of dry DMF was treated with 60% NaH in mineral oil (0.23 g, 5.8 mmol). After stirring for 50 min at room temperature, the resulting solution was treated with CH_3I (0.35 mL, 5.6 mmol) for 1 h, then acidified with acetic acid, and filtered. The filtrate was concentrated to give an oil which was dissolved in CH_2Cl_2, washed once with H_2O, twice with 5% Na_2CO_3, and once with H_2O, then dried, and concentrated to give 1.33 g (85%) of 5.

[4-(Methylamino)-3-nitrophenyl](2-thienyl)methanone (31). Methylamine was bubbled into a solution of 30[3] (10.0 g, 0.0374 mol) in 100 mL of dry DMSO for 2.25 h. The solution was poured into ice/H_2O, producing a precipitate which was filtered, washed with H_2O, and vacuum dried to give 9.45 g (96%) of 31: mp 148–149.5 °C.

Methyl [1-Methyl-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (6). A suspension of 31 (2.29 g, 8.73 mmol) in 200 mL of MeOH containing 0.5 g of 10% Pd/C was hydrogenated on a Parr shaker for 3.5 h at 65 °C. The cooled solution was degassed, filtered, acidified with ethereal HCl, and concentrated to dryness, leaving 2.28 g (97%) of diamine hydrochloride: mp 166–170 °C (CH_3CN).

A suspension of 2-methyl-2-thiopseudourea sulfate (9.23 g, 0.0332 mol), 15 mL of H_2O, and methyl chloroformate (5.14 mL, 0.0665 mol) was cooled in an ice bath and 25% NaOH was added until the pH stabilized at approximately 8. The pH was then lowered to 5 with acetic acid; sodium acetate trihydrate (4.51 g, 0.0332 mol) was added to give a solution of 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea. This solution was then allowed to react with a warm solution of diamine hydrochloride (8.92 g, 0.0332 mol) in 87 mL of dioxane and 148 mL of H_2O. The reaction mixture was heated at reflux for 1 h during which time a precipitate formed. The cooled mixture was filtered and the precipitate was washed with H_2O and dried to give 5.36 g (51%) of 6.

(3-Methoxy-4-nitrophenyl)(2-thienyl)methanone (33). A suspension of 32 (50.0 g, 0.254 mol), thionyl chloride (60.0 mL, 0.823 mol), $CHCl_3$ (120 mL), and DMF (5 drops) was heated at reflux for several hours under nitrogen until a solution formed and then concentrated to dryness at 60 °C. The resulting solid acid chloride was dissolved in 166 mL of CH_2Cl_2 and added in a slow stream at below 10 °C with ice bath cooling to a suspension of $AlCl_3$ (33.9 g, 0.254 mol) in 166 mL of CH_2Cl_2 to give a solution. Thiophene (22.4 mL, 0.279 mol) was then added dropwise at below 10 °C. The reaction mixture was stirred for 0.5 h in the cold and then 2 h at room temperature and was poured into ice/H_2O. The organic layer was washed twice with 5% $NaHCO_3$, dried with $MgSO_4$ containing decolorizing charcoal, then filtered, and con-

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(11) (a) Roach, M. C.; Ludueña, R. F. *J. Biol. Chem.* **1984**, *259*, 12063. (b) Little, M.; Ludueña, R. F. *EMBO J.* **1985**, *4*, 51.

(12) Still, W. C.; Kalin, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

centrated. Recrystallization from toluene afforded 28.9 g (43%) of **33**: mp 117–118 °C.

[3-(Methylamino)-4-nitrophenyl](2-thienyl)methanone (34). Methylamine was bubbled for 8.5 h into a solution of **33** (5.0 g, 0.019 mol) in 50 mL of dry DMSO which was heated in an oil bath at 85 °C. The cooled reaction mixture was poured into ice/H_2O and was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed three times with H_2O, dried, and concentrated. Recrystallization from MeOH afforded 3.31 g (67%) of **34**: mp 114.5–116 °C.

Methyl [3-Methyl-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (7). Four 3.0-g batches of **34** (12.0 g total, 0.0458 mol) were each reduced in 200 mL of hot MeOH containing 0.5 g of 10% Pd/C for 1 h as in the preparation of **6**. Reaction of the diamine hydrochloride with a solution of 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea prepared as for **6** (dioxane omitted) followed by EtOAc extraction afforded 14.6 g of crude **7**. Chromatography (EtOAc) followed by two recrystallizations from acetone yielded 0.30 g of **7**.

3-Methoxy-2-methylbenzoic Acid (37). Oxazoline **36** was methylated by using the literature procedure[7] to afford 89% of methylated intermediate: bp 76–81.5 °C (0.17–0.13 Torr). A mixture of 20.0 g (0.0912 mol) of methylated oxazoline and 1.0 L of 5 N HCl was heated at reflux for 12 h. The cooled reaction mixture was extracted twice with $CHCl_3$. The $CHCl_3$ extracts were extracted twice with 5% $NaHCO_3$, and the combined $NaHCO_3$ extracts were acidified with concentrated HCl. The precipitated white solid was washed with H_2O, dried, and recrystallized from toluene to give 9.12 g (60%) of **37**: mp 146.5–149 °C.

(3-Methoxy-2-methyl-4-nitrophenyl)(2-thienyl)methanone (38a). Reaction of **37** (12.9 g, 0.0775 mol) with thionyl chloride (15.5 mL, 0.212 mol), then $AlCl_3$ (10.3 g, 0.0775 mol), and thiophene (6.79 mL, 0.0849 mol) as in the preparation of **33** afforded 10.9 g (60%) of ketone: bp 116–122 °C (0.15 Torr).

A solution of the above ketone (10.7 g, 0.046 mol) in 390 mL of CH_2Cl_2 was cooled in an ice bath and treated successively with concentrated sulfuric acid (11.5 mL) and concentrated nitric acid (3.24 mL, 0.05 mol). The reaction mixture was stirred in the cold for 2 h and then excess 5% $NaHCO_3$ was added cautiously. The organic layer was washed with 5% $NaHCO_3$, then dried, and concentrated. Recrystallization from MeOH afforded 8.92 g (70%) of **38a** in two crops: mp 99–101.5 °C.

(4-Methoxy-2-methylphenyl)(2-thienyl)methanone (40a). A mixture of thiophene-2-carboxylic acid (41.9 g, 0.327 mol), thionyl chloride (93 mL, 1.3 mol), and 233 mL of $CHCl_3$ was heated at reflux overnight under nitrogen and then concentrated to give thiophene-2-carbonyl chloride (**39**) as a liquid. A solution of 3-methylanisole (43.9 g, 0.36 mol) in 78 mL of CH_2Cl_2 was added slowly at below 12 °C with ice bath cooling to a suspension of $AlCl_3$ (65.5 g, 0.491 mol) in 186 mL of CH_2Cl_2. A solution of the above acid chloride in 100 mL of CH_2Cl_2 was slowly added at below 12 °C. The dark mixture was stirred for 45 min in the cold and then cautiously poured into ice/H_2O. The organic layer was washed twice with 5% $NaHCO_3$, then dried, and concentrated to give 76.0 g of an oil (mixture of isomers). Vacuum distillation of 20.0 g of this oil produced 12.9 g of distillate [bp 124–140 °C (0.5–0.7 Torr)] which was further purified by chromatography (hexane containing 33–100% CH_2Cl_2) to afford 5.91 g (30%) of **40a** as an oil; 0.6 g (3%) of the isomeric (2-methoxy-4-methylphenyl)(2-thienyl)methanone eluted later: mp 70.5–72 °C.

(4-Methoxy-3-methylphenyl)(2-thienyl)methanone (40b). The reaction of 2-methylanisole (43.9 g, 0.36 mol), **39** (0.327 mol), and $AlCl_3$ (65.5 g, 0.491 mol) as in the preparation of **40a** yielded 75.2 g (99%) of **40b**: mp 56–59 °C; vacuum distillation [bp 148–153 °C (0.35 Torr)] raised the mp to 61–63.5 °C.

(4-Methoxy-2-methyl-5-nitrophenyl)(2-thienyl)methanone (38b). The reaction of **40a** (12.8 g, 0.0552 mol), concentrated sulfuric acid (13.8 mL), and concentrated nitric acid (3.89 mL, 0.0608 mol) as in the preparation of **38a** yielded 16.1 g of nitrated products. Chromatography (CH_2Cl_2) afforded 6.18 g (40%) of **38b**. Analytically pure **38b** was obtained after two recrystallizations from MeOH followed by sublimation (100–115 °C, 0.08 Torr): mp 135–137 °C.

(4-Methoxy-3-methyl-5-nitrophenyl)(2-thienyl)methanone (38c). A 1.0 M solution of BBr_3 in CH_2Cl_2 (33.1 mL, 0.0331 mol) was added dropwise under nitrogen with ice bath cooling to a solution of **40b** (3.85 g, 0.0166 mol) in 40 mL of CH_2Cl_2. After stirring for 30 min in the cold and for 5 h at room temperature, the solution was cooled in an ice bath and slowly treated with excess MeOH. The reaction mixture was then concentrated to dryness; the residue was redissolved in MeOH and concentrated. The residue was dissolved in dilute NaOH, washed with Et_2O, and acidified with concentrated HCl. The precipitated solid was washed with H_2O and dried to give 3.45 g (95%) of phenol: mp 159.5–164 °C. The phenol (5.12 g, 0.0235 mol) was nitrated with concentrated sulfuric acid (5.85 mL) and concentrated nitric acid (1.66 mL, 0.0259 mol) as in the preparation of **38a** to yield 5.95 g (96%) of nitrophenol: mp 121–123.5 °C (EtOH). A mixture of nitrophenol (5.95 g, 0.0226 mol), dimethyl sulfate (5.35 mL, 0.0565 mol), K_2CO_3 (7.80 g, 0.0565 mol), and acetone was heated at reflux for 12 h under nitrogen. The reaction mixture was cooled and diluted with H_2O and then extracted three times with Et_2O. The Et_2O extracts were washed twice with H_2O and then stirred for 1.25 h with a 1.5 N solution of NH_4OH. The organic layer was washed twice with H_2O, then dried, and concentrated. The product was purified by chromatography (1:3 EtOAc/hexane) followed by recrystallization from EtOH to give 2.95 g (47%) of **38c**: mp 102–103.5 °C.

Methyl [4-Methyl-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (8). Anhydrous NH_3 was bubbled for 76 h into a heated (105 °C) solution of **38a** (8.15 g, 0.0294 mol) in 82 mL of dry DMSO. The cooled reaction mixture was poured into H_2O and extracted three times with Et_2O. The combined Et_2O extracts were washed three times with H_2O, then dried, and concentrated to a dark oil. The oil was purified by flash chromatography (CH_2Cl_2) to yield 4.16 g (54%) of bright yellow solid: mp 162.5–164 °C. A solution of the above solid (2.0 g, 7.63 mmol) in 200 mL of MeOH containing 0.4 g of 10% Pd/C was hydrogenated for 1.25 h at 50 °C. The mixture was cooled, degassed, filtered, and concentrated. The residual diamine was dissolved in 40 mL of MeOH and treated with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (1.64 g, 7.95 mmol) and a catalytic amount of *p*-toluenesulfonic acid. The mixture was heated at reflux for 10 min during which time a precipitate formed. The precipitated **8** was isolated from the cooled reaction mixture by filtration, then washed with MeOH, and dried to yield 1.48 g (62%) of **8**.

Methyl [6-Methyl-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (9). Reaction of **38b** (3.0 g, 0.0108 mol) with NH_3 in DMSO for 14 h at 85 °C as in the preparation of **8** yielded after chromatography (CH_2Cl_2) 1.68 g (59%) of yellow solid: mp 163–165 °C. This solid (1.77 g, 6.75 mmol) was hydrogenated over 0.5 g of 10% Pd/C for 2.5 h at 50 °C and the resulting diamine was reacted with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (1.46 g, 7.09 mmol) and *p*-toluenesulfonic acid as in the preparation of **8** to yield 1.88 g (88%) of **9**.

Methyl [7-Methyl-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (10). Reaction of **38c** (2.8 g, 0.01 mol) with NH_3 in DMSO for 3 h at 85 °C as in the preparation of **8** yielded 2.59 g (98%) of yellow-orange solid: mp 125–128.5 °C. This solid (2.33 g, 8.88 mmol) was hydrogenated over 0.5 g of 10% Pd/C for 2 h at 50 °C, and the resulting diamine was reacted with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (1.92 g, 9.33 mmol) as described in the preparation of **8**, to yield 2.29 g (82%) of **10**.

(4-Methoxyphenyl)(3-methyl-2-thienyl)methanone (44a). Reaction of **42a**[13] (5.0 g, 0.0352 mol), thionyl chloride (7.85 mL, 0.108 mol), and $CHCl_3$ (25 mL) for 5 h as in the preparation of **40a** yielded **43a** as an amber liquid. Reaction of anisole (4.19 g, 0.0387 mol), $AlCl_3$ (7.04 g, 0.0528 mol), and **43a** (0.0352 mol) for 25 min as in the preparation of **40a** yielded after distillation [bp 142–150 °C (0.3 Torr)] 6.0 g (73%) of **44a**: mp 53–56.5 °C.

(4-Methoxy-3-nitrophenyl)(3-methyl-2-thienyl)methanone (45a). An acetic anhydride (338 mL) solution of **44a** (49.4 g, 0.213 mol) was cooled below 15 °C in an ice bath and treated dropwise with concentrated nitric acid (14.3 mL, 0.223 mol). The resulting yellow solution was stirred in the cold for 1.25 h and then concentrated to give an oil. An Et_2O solution of this oil was washed thoroughly with 5% $NaHCO_3$, followed by 5% Na_2CO_3, then dried,

(13) Available from the Aldrich Chemical Co., Inc., Milwaukee, IL.

and concentrated to a syrup. Upon refrigeration the syrup deposited crystals which were purified by recrystallization from EtOH to yield 2.17 g of **45a**: mp 112–117.5 °C. After chromatography (hexane containing 50–100% CH_2Cl_2, followed by 1% EtOAc in CH_2Cl_2) and recrystallization from MeOH, the mother liquors provided an additional 7.25 g of **45a**: mp 106–116 °C for a total yield of 9.42 g (16%).

(4-Methoxyphenyl)(4-methyl-2-thienyl)methanone (44b). Reaction of **42b**[14] (6.57 g, 0.0462 mol), thionyl chloride (13.1 mL, 0.180 mol), and $CHCl_3$ (33 mL) for 4 h as in the preparation of **40a** yielded **43b**. Reaction of anisole (5.5 g, 0.0509 mol), $AlCl_3$ (9.25 g, 0.0694 mol), and **43b** (0.0462 mol) for 30 min as in the preparation of **40a** yielded after distillation [bp 134–138 °C (0.2 Torr)] 7.0 g (65%) of **44b**.

(4-Methoxy-3-nitrophenyl)(4-methyl-2-thienyl)methanone (45b). Reaction of **44b** (37.0 g, 0.159 mol), acetic anhydride (253 mL), and concentrated nitric acid (10.7 mL, 0.167 mol) as in the preparation of **45a** followed by chromatography (hexane containing 25–50% EtOAc) and recrystallization from MeOH yielded 2.17 g (4.9%) of **45b**: mp 114–119 °C.

(4-Chloro-3-nitrophenyl)(5-methyl-2-thienyl)methanone (47). Reaction of 4-chloro-3-nitrobenzoyl chloride (30.4 g, 0.138 mol) [from 4-chloro-3-nitrobenzoic acid (27.8 g, 0.138 mol), thionyl chloride (33.4 mL, 0.458 mol), $CHCl_3$ (67 mL) and DMF (6 drops)], $AlCl_3$ (18.4 g, 0.138 mol), and **46** (14.7 mL, 0.152 mol) as in the preparation of **33** yielded 17.1 g (44%) of **47** after recrystallization from toluene/hexane: mp 139.5–143 °C.

(4-Amino-3-nitrophenyl)(5-methyl-2-thienyl)methanone (48c). Reaction of **47** (10.0 g, 0.0355 mol) with NH_3 in DMSO for 4.75 h at 85 °C as in the preparation of 8 yielded 8.18 g (88%) of **48c**: mp 134–137.5 °C.

(4-Amino-3-nitrophenyl)(3-methyl-2-thienyl)methanone (48a). Reaction of **45a** (8.56 g, 0.0309 mol) with NH_3 in DMSO for 11 h at 85 °C as in the preparation of 8 yielded 5.21 g (64%) of **48a** after recrystallization from MeOH: mp 151–153 °C.

(4-Amino-3-nitrophenyl)(4-methyl-2-thienyl)methanone (48b). Reaction of **45b** (2.78 g, 0.01 mol) with NH_3 in DMSO for 13 h at 85 °C as in the preparation of 8 yielded 2.37 g (90%) of **48b**: mp 163–166.5 °C.

Methyl [5-[(3-Methyl-2-thienyl)carbonyl]-1*H*-benzimidazol-2-yl]carbamate (11). Two 2.0-g batches of **48a** (4.0 g total, 0.0153 mol) were each reduced over 0.5 g of 10% Pd/C in 200 mL of hot MeOH for 3 h at 50 °C as in the preparation of 8. Reaction of the resulting diamine, MeOH (80 mL), 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (3.32 g, 0.0161 mol), and *p*-toluenesulfonic acid as in the preparation of 8 yielded 2.81 g (59%) of **11** after recrystallization from acetic acid/H_2O.

Methyl [5-[(4-Methyl-2-thienyl)carbonyl]-1*H*-benzimidazol-2-yl]carbamate (12). Reduction of **48b** (2.27 g, 8.66 mmol) as in the preparation of 11 followed by reaction of the diamine intermediate in MeOH with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (1.87 g, 9.09 mmol) and *p*-toluenesulfonic acid as in the preparation of 8 yielded 2.19 g (80%) of **12**.

Methyl [5-[(5-Methyl-2-thienyl)carbonyl]-1*H*-benzimidazol-2-yl]carbamate (13). Reduction of **48c** (4.0 g, 0.0153 mol) in two batches as in the preparation of **6**, followed by reaction of the diamine hydrochloride with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea for 50 min at 85 °C as in the preparation of **6** (dioxane omitted), yielded 2.53 g (52%) of **13** after recrystallization from acetic acid/H_2O.

[4-[[2-[(Benzyloxycarbonyl)amino]ethyl]amino]-3-nitrophenyl](2-thienyl)methanone (50a). A solution of **30** (8.74 g, 0.0327 mol), **49a**[15] (6.98 g, 0.0359 mol), and Et_3N (7.51 mL, 0.0539 mol) in DMSO (88 mL) was stirred at room temperature for 44 h. The mixture was diluted with cold H_2O and extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed three times with H_2O, then dried, and concentrated. Recrystallization of the crude product from toluene afforded 10.4 g (75%) of **50a**: mp 113–114.5 °C.

[4-[[2-[(*tert*-Butoxycarbonyl)amino]ethyl]amino]-3-nitrophenyl](2-thienyl)methanone (50b). Reaction of **30** (7.26 g, 0.0271 mol), **49b**[16] (5.0 g, 0.0312 mol), and Et_3N (6.24 mL, 0.0448 mol) in DMSO (73 mL) for 48 h as in the preparation of **50a** yielded 6.95 g (65%) of **50b** after chromatography (1:2 EtOAc/hexane followed by 1:1 EtOAc/hexane): mp 132–135 °C.

[4-[[4-[(*tert*-Butoxycarbonyl)amino]butyl]amino]-3-nitrophenyl](2-thienyl)methanone (50c). Reaction of **30** (6.09 g, 0.0227 mol), **49c**[17] (4.71 g, 0.025 mol), and Et_3N (5.23 mL, 0.0375 mol) in DMSO (61 mL) for 45 h as in the preparation of **50a** yielded 5.08 g (53%) of **50c** after trituration with boiling Et_2O: mp 94–96 °C.

Methyl [1-[2-[(Benzyloxycarbonyl)amino]ethyl]-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (14). Hydrogenation of **50a** (2.30 g, 5.41 mmol) over Pt_2O (0.3 g) for 3.3 h at 50 °C followed by reaction of the resulting diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (1.23 g, 5.95 mmol) and *p*-toluenesulfonic acid as described in the preparation of 8 yielded 2.15 g (83%) of **14**.

Methyl [1-[2-[(*tert*-Butoxycarbonyl)amino]ethyl]-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (51a). Hydrogenation of **50b** (4.0 g, 0.0102 mol) over 10% Pd/C for 3 h at 50 °C followed by reaction of the diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (2.21 g, 0.0107 mol) and *p*-toluenesulfonic acid for 2 h as described in the preparation of 8 afforded a solution of **51a**. Concentration of the solution followed by chromatography (1:1 Et_2O/CH_2Cl_2, then Et_2O, followed by EtOAc) and recrystallization from EtOAc/hexane yielded 4.04 g (89%) of **51a**: mp 170–173 °C dec.

Methyl [1-[4-[(*tert*-Butoxycarbonyl)amino]butyl]-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate (51b). Hydrogenation of **50c** (1.67 g, 3.98 mmol) over 0.5 g of 10% Pd/C for 2 h at 55 °C followed by reaction of the resulting diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (0.86 g, 4.2 mmol) and *p*-toluenesulfonic acid as described in the preparation of **51a** yielded 1.67 g (89%) of **51b**: mp 92–94 °C.

Methyl [1-(2-Aminoethyl)-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate Diformate (15·2HCO_2H). A solution of **51a** (1.0 g, 2.25 mmol) in 30 mL of formic acid was stirred for 16 h at room temperature and then concentrated at 30 °C to yield 0.93 g (95%) of 15·2HCO_2H.

Methyl [1-(4-Aminobutyl)-5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate Acetate (16·CH_3CO_2H). A solution of **51b** (1.52 g, 3.22 mmol) in formic acid (47 mL) was stirred at room temperature for 3 h and then concentrated at 30 °C to yield an oil. The oil was made basic with aqueous $NaHCO_3$ and the mixture was extracted three times with $CHCl_3$. The $CHCl_3$ extracts were dried and concentrated to a yellow foam which was dissolved in $CHCl_3/Et_2O$ and acidified with AcOH to give 0.79 g (57%) of 16·CH_3CO_2H as a yellow solid.

[4-(Cyanomethyl)-2-thienyl](3,4-dinitrophenyl)methanone (53). A mixture of 3,4-dinitrobenzoic acid (46.7 g, 0.22 mol) and thionyl chloride (80.0 mL, 1.1 mol) was heated at reflux for 12 h and then concentrated at 60 °C to yield the acid chloride as an oil. A solution of the acid chloride in CH_2Cl_2 (200 mL) was slowly added at room temperature to a slurry of $AlCl_3$ (66.7 g, 0.5 mol) in CH_2Cl_2 (200 mL) followed by the addition of a solution of **52** (24.6 g, 0.2 mol) in CH_2Cl_2 (100 mL). After heating at reflux for 2.5 h, the mixture was cooled in an ice bath and the liquid phase was decanted into ice/H_2O. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 solutions were washed successively with H_2O and 5% $NaHCO_3$, then dried, and concentrated to an oil. Crystallization from hot EtOH yielded 1.63 g of product: mp 154–156 °C. Treatment of the decantation residue with water with cooling yielded a solid which was filtered, washed with H_2O, and dried to give 55.4 g of **53** (total yield 57.0 g, 90%).

Methyl [5-[[4-(Cyanomethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (21). Two 0.5-g portions of **53** (1.0 g total, 3.15 mmol) were each dissolved in 200 mL of hot MeOH and then hydrogenated over 0.1 g of 10% Pd/C at ambient

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temperature for 45 min. The mixtures were degassed, filtered, combined, and concentrated. The resulting diamine was then reacted with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (0.68 g, 3.3 mmol) and *p*-toluenesulfonic acid in MeOH (15 mL) as in the preparation of **8** to yield 0.96 g (90%) of **21**.

[4-(Carboxymethyl)-2-thienyl](3,4-dinitrophenyl)methanone (54). A mixture of **53** (10.9 g, 0.0343 mol) and 45% H_2SO_4 (71 mL) was heated at reflux for 4.5 h. The cooled reaction mixture was diluted with H_2O and filtered. The precipitate was washed with H_2O, dried, and recrystallized from toluene to yield 8.98 g (78%) of **54**: mp 159.5–161.5 °C.

Methyl [5-[[4-(Carbomethoxymethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (17). Hydrogen chloride was bubbled into a suspension of **54** (2.13 g, 6.33 mmol) in MeOH (50 mL) to give a warm solution which was then heated at reflux for 1.5 h. The solution was concentrated and the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed succesively with H_2O and 5% $NaHCO_3$, then dried, and concentrated to yield an oil. The oil was purified by chromatography (CH_2Cl_2) to yield 1.17 g (53%) of ester. Hydrogenation of this dinitro ester (1.08 g, 3.08 mmol) in MeOH (200 mL) over 0.4 g of 10% Pd/C for 2 h at ambient temperature followed by reaction of the resulting diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (0.67 g, 3.2 mmol) and *p*-toluenesulfonic acid in MeOH (20 mL) as in the preparation of **21** yielded 0.9 g (78%) of **17**.

[4-(Carboxymethyl)-2-thienyl](4-chloro-3-nitrophenyl)methanone (56a). Reaction of 4-chloro-3-nitrobenzoyl chloride (0.11 mol) [from 22.2 g (0.11 mol) of 4-chloro-3-nitrobenzoic acid, thionyl chloride (27.0 mL, 0.37 mol), $CHCl_3$ (53 mL) and DMF (trace)], $AlCl_3$ (29.3 g, 0.22 mol), and **55a** (14.2 g, 0.1 mol) for 1.5 h at reflux as in the preparation of **33** resulted in the formation of a gum. The cooled CH_2Cl_2 solution was decanted into ice/H_2O. The gum was slowly treated with H_2O and then extracted with CH_2Cl_2. The CH_2Cl_2 extract was added to the decanted aqueous/CH_2Cl_2 solution, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 solutions were washed with H_2O, dried, and concentrated to 35.8 g of crude **56a**.

[4-(Carbo-*tert*-butoxymethyl)-2-thienyl](4-chloro-3-nitrophenyl)methanone (57). A solution of crude **56a** (21.1 g, 0.0589 mol) and thionyl chloride (21.1 mL, 0.289 mol) in CH_2Cl_2 (211 mL) was stirred for 18 h under argon, and the resulting dark solution was concentrated without heating. The residual acid chloride was dissolved in CH_2Cl_2 (127 mL) and added over a period of 30 min to a solution of *tert*-butyl alcohol (76.5 mL, 0.811 mol) and Et_3N (15.8 mL, 0.114 mol) in CH_2Cl_2 (317 mL) with ice bath cooling under argon. After stirring in the cold for 1 h, the mixture was concentrated and the residue was dissolved in EtOAc, washed with 5% $NaHCO_3$, dried, and concentrated. The crude product (15.5 g) was purified by chromatography (CH_2Cl_2) to yield 5.66 g (25% from **55a**) of **57**: mp 109–114 °C.

Methyl [5-[[4-(Carbo-*tert*-butoxymethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (19). Reaction of **57** (6.32 g, 0.0166 mol) with NH_3 in DMSO for 6 h at 85 °C as in the preparation of **8** followed by chromatography (CH_2Cl_2 with 0–10% Et_2O gradient) yielded 3.57 g (60%) of nitroaniline intermediate: mp 122.5–124 °C. The nitroaniline (3.51 g, 9.69 mmol) was dissolved in warm MeOH (200 mL) and hydrogenated over 0.5 g of 10% Pd/C for 2 h at ambient temperature as in the preparation of **8**. The resulting diamine was reacted with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (2.10 g, 10.17 mmol) and *p*-toluenesulfonic acid as in the preparation of **8** to yield 3.16 g (79%) of **19**.

[4-(Carbethoxymethyl)-2-thienyl](4-chloro-3-nitrophenyl)methanone (56b). Reaction of 4-chloro-3-nitrobenzoyl chloride (4.84 g, 0.022 mol), $AlCl_3$ (5.87 g, 0.044 mol), and **55b** (3.40 g, 0.02 mol) as in the preparation of **33** yielded 7.0 g of a tan oil which was purified by chromatography (CH_2Cl_2) to give 4.99 g (70%) of **56b** as an oil.

Methyl [5-[[4-(Carbethoxymethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (18). Reaction of **56b** (4.97 g, 0.014 mol) with NH_3 in DMSO for 4.5 h at 85 °C as in the preparation of **8** yielded after chromatography (1:19 Et_2O/CH_2Cl_2) 2.64 g (56%) of nitroaniline intermediate: mp 87–88.5 °C. Hydrogenation of a warm solution of the nitroaniline (2.64 g, 7.9 mmol) for 1.5 h in EtOH (200 mL) over 0.5 g of 10% Pd/C followed by reaction of the diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (1.71 g, 8.29 mmol) and *p*-toluenesulfonic acid as in the preparation of **8** yielded 2.23 g (73%) of **18**.

Methyl [5-[[4-(Carboxymethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (20). A solution of **19** (0.656 g, 1.58 mmol) in formic acid (13.1 mL) was stirred at room temperature under argon for 26 h and then concentrated without heating. The residue was triturated with Et_2O and then recrystallized from acetic acid to yield 0.181 g (32%) of **20**.

[4-[[(*tert*-Butoxycarbonyl)amino]methyl]-2-thienyl](3,4-dinitrophenyl)methanone (58). A mixture of **54** (3.48 g, 0.0104 mol) and thionyl chloride (16.5 mL, 0.226 mol) was heated at reflux for 3 h and then concentrated at 65 °C. The residual acid chloride, toluene (60 mL), and azidotrimethylsilane (3.0 mL, 0.0226 mol) were heated at reflux for 16 h under argon. The hot reaction mixture was decanted and concentrated. The concentrate was heated at reflux for 6 h with *tert*-butyl alcohol (60 mL), then concentrated, and chromatographed (CH_2Cl_2 containing 0–10% Et_2O) to yield 2.90 g (69%) of **58**: mp 139–141.5 °C.

Methyl [5-[[4-[[(*tert*-Butoxycarbonyl)amino]methyl]-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (23). Hydrogenation of **58** (1.045 g, 2.565 mmol) in MeOH (200 mL) over 0.2 g of 10% Pd/C for 45 min at ambient temperature followed by reaction of the resulting diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (0.555 g, 2.69 mmol) and *p*-toluenesulfonic acid as in the preparation of **8** yielded 0.804 g (73%) of **23**.

Methyl [5-[[4-(Aminomethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate Dihydrochloride (24). A solution of **23** (0.5 g, 1.16 mmol) in formic acid (10 mL) was stirred for 4 h at ambient temperature under argon. Most of the formic acid was removed by concentration below 35 °C, leaving a syrup. The syrup was diluted with H_2O and then made basic by the addition of excess $NaHCO_3$ to give a precipitate which was filtered and washed with H_2O. The precipitate was suspended in MeOH and treated with an excess of ethereal HCl. The resulting solution was then concentrated to an oil which was recrystallized from MeOH/Et_2O to yield 0.419 g (83%) of **24**.

(3,4-Dinitrophenyl)[4-(hydroxymethyl)-2-thienyl]methanone (59). A mixture of **58** (1.61 g, 3.95 mmol) and 43.5 mL of 3 M HCl in EtOAc was stirred at room temperature for 0.5 h to give a suspension which was diluted with Et_2O. The precipitated product was filtered and washed with Et_2O to yield 1.33 g (98%) of amine hydrochloride: mp 204 °C dec. The amine hydrochloride (2.43 g, 7.07 mmol) was added to a solution of sodium nitrite (0.976 g, 14.1 mmol) in a mixture of DMSO (48.6 mL) and H_2O (4.9 mL) followed by the addition of *p*-toluenesulfonic acid hydrate (2.69 g, 14.1 mmol). The reaction mixture was stirred under argon for 10 min at room temperature followed by heating in an oil bath at 100 °C for 1 h. The cooled reaction mixture was diluted with H_2O and extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed twice with H_2O, dried, concentrated, and chromatographed (CH_2Cl_2 with 0–15% Et_2O gradient) to yield 0.88 g (40%) of **59**: mp 138–145 °C.

Methyl [5-[[4-(Hydroxymethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (27). Hydrogenation of **59** (0.1 g, 0.331 mmol) in MeOH (50 mL) over 0.02 g of 10% Pd/C for 45 min at room temperature followed by reaction of the resulting diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (0.075 g, 0.364 mmol) and *p*-toluenesulfonic acid as in the preparation of **8** yielded 72.8 mg (66%) of **27**.

[4-(Carbomethoxymethyl)-2-thienyl](4-chloro-3-nitrophenyl)methanone (60). Reaction of **56a** (15.8 g, 0.0485 mol) with hydrogen chloride in MeOH (500 mL) as in the preparation of **17** yielded after chromatography (CH_2Cl_2) 12.3 g (75%) of **60**: mp 74–75.5 °C (MeOH).

(4-Amino-3-nitrophenyl)[4-[[*N*-[2-[(*tert*-butoxycarbonyl)amino]ethyl]carbamoyl]methyl]-2-thienyl]methanone (61). Reaction of **60** (8.58 g, 0.0253 mol) with NH_3 in DMSO for 3.5 h at 85 °C as in the preparation of **8** yielded 3.49 g (43%) of nitroaniline intermediate: mp 136.5–141 °C. A mixture of nitroaniline (3.45 g, 0.0108 mol), **49b** (3.45 g, 0.0215 mol), and 2-hydroxypyridine (0.77 g, 0.0081 mol) was heated in an oil bath at 120 °C under argon for 2 h. The cooled reaction

mixture was chromatographed (EtOAc) to give 3.58 g of a yellow solid which was further purified by trituration with $CHCl_3$ followed by recrystallization from MeOH to yield 1.96 g (41%) of **61**: mp 180–182 °C.

Methyl [5-[[4-[[*N*-(2-Aminoethyl)carbamoyl]methyl]-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate Formate (22). Hydrogenation of **61** (1.3 g, 2.9 mmol) in MeOH (130 mL) over 0.26 g of 10% Pd/C for 1.5 h at 50 °C followed by reaction of the resulting diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (0.63 g, 3.1 mmol) and *p*-toluenesulfonic acid as in the preparation of **8** yielded 1.26 g (87%) of the intermediate BOC-protected product. The BOC protecting group was removed by treating 1.22 g (2.43 mmol) of intermediate with 100 mL of formic acid for 2 h at ambient temperature. Removal of the formic acid at 30 °C left an oil which upon trituration with MeOH produced 0.679 g (62%) of crystalline **22**.

[4-(Benzoyloxymethyl)-2-thienyl](3,4-dinitrophenyl)-methanone (62). A mixture of **59** (0.2 g, 0.65 mmol), benzoic acid (79 mg, 0.65 mmol), 4-pyrrolidinopyridine (9.6 mg, 0.065 mmol), dicyclohexylcarbodiimide (0.15 g, 0.73 mmol), dioxane (2.5 mL), and CH_2Cl_2 (2.5 mL) was stirred at ambient temperature for 5 h under argon and then filtered. The filtrate was concentrated and chromatographed (CH_2Cl_2) to yield 71 mg (27%) of **62**.

Methyl [5-[[4-(Benzoyloxymethyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (25). A solution of **62** (71 mg, 0.17 mmol) in THF (5 mL) was treated with H_2O (5 mL) and then technical grade (85%) sodium hydrosulfite (0.70 g, 3.4 mmol). The reaction mixture was stirred at ambient temperature under argon for 26 h and extracted twice with CH_2Cl_2. The combined extracts were washed twice with H_2O, dried, and concentrated. The residual diamine was reacted with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (39 mg, 0.19 mmol) and *p*-toluenesulfonic acid in MeOH as in the preparation of **8** to yield 9.2 mg (12%) of **25**.

[4-(Carbobenzyloxymethyl)-2-thienyl](3,4-dinitrophenyl)methanone (63). A mixture of **54** (1.68 g, 5.0 mmol), benzyl alcohol (0.595 g, 5.5 mmol), 4-pyrrolidinopyridine (0.074 g, 0.5 mmol), dicyclohexylcarbodiimide (1.13 g, 5.5 mmol), dioxane (7.5 mL), and CH_2Cl_2 (15 mL) was reacted for 2.75 h as described in the preparation of **62** to yield 1.43 g (67%) of **63**.

Methyl [5-[[4-(Carbobenzyloxymethyl)-2-thienyl]-carbonyl]-1*H*-benzimidazol-2-yl]carbamate (26). A solution of **63** (0.242 g, 0.568 mmol) in THF (10 mL) was treated with H_2O (10 mL) and then technical grade (85%) sodium hydrosulfite (2.0 g, 9.8 mmol). The reaction mixture was stirred at room temperature for 18 h, and the layers were separated. The aqueous layer was extracted with THF and the THF extract was combined with the organic layer and washed with a solution of saturated NaCl. The solution was dried and concentrated. Reaction of the residual diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (0.129 g, 0.624 mmol) and *p*-toluenesulfonic acid in MeOH as in the preparation of **8** produced a solution. The solution was concentrated to an oil which then yielded 42.6 mg (17%) of crystalline **26** upon trituration with MeOH; the analytical sample was recrystallized from EtOAc/hexane.

Methyl [5-[[4-(Trifluoroacetyl)-2-thienyl]carbonyl]-1*H*-benzimidazol-2-yl]carbamate (28). Reaction of **64**[5] (0.202 g, 0.562 mmol) with NH_3 in DMSO (5 mL) for 4 h and 40 min as in the preparation of **8** yielded after chromatography (CH_2Cl_2) 84 mg (43%) of the nitroaniline. Hydrogenation of the nitroaniline (84 mg, 0.24 mmol) in MeOH (50 mL) over 20 mg of 10% Pd/C for 2.25 h at ambient temperature followed by reaction of the diamine with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea (55.3 mg, 26.8 mmol) and *p*-toluenesulfonic acid for 25 min as in the preparation of **8** produced a solution. Concentration and trituration with Et_2O/CH_2Cl_2 yielded 27.4 mg (28%) of **28**.

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Registry No. **1**, 31430-18-9; **2**, 61167-21-3; **3**, 61167-12-2; **4**, 61167-13-3; **5**, 117498-12-1; **6**, 117498-13-2; **7**, 117498-14-3; **8**, 117498-15-4; **9**, 117498-16-5; **10**, 117498-17-6; **11**, 117498-18-7; **12**, 117498-19-8; **13**, 66939-18-2; **14**, 117498-20-1; **15**, 117498-21-2; **15**·2HCO_2H, 117498-22-3; **16**, 117498-23-4; **16**·CH_3CO_2H, 117498-24-5; **17**, 117498-25-6; **18**, 117498-26-7; **19**, 117498-27-8; **20**, 117498-28-9; **21**, 117498-29-0; **22**, 117498-30-3; **22**, BOC-protected derivative, 117498-97-2; **23**, 117498-31-4; **24**, 117498-92-7; **24**·2HCl, 117498-32-5; **25**, 117498-33-6; **26**, 117498-34-7; **27**, 117498-35-8; **28**, 117498-36-9; **29**, 117498-37-0; **30**, 31431-18-2; **31**, 117498-38-1; **31**·HCl, diamine derivative, 117498-57-4; **32**, 5081-36-7; **32**, acid chloride derivative, 67579-92-4; **33**, 117498-39-2; **34**, 117498-40-5; **34**·HCl, diamine derivative, 117498-58-5; **35**, 586-38-9; **36**, 73453-77-7; **36**, methylated derivative, 72623-17-7; **37**, 55289-06-0; **38a**, 117498-41-6; **38a**, amine derivative, 117498-64-3; **38a**, denitro derivative, 117498-59-6; **38a**, diamine derivative, 117498-65-4; **38b**, 117498-61-0; **38b**, amine derivative, 117498-66-5; **38b**, diamine derivative, 117498-67-6; **38c**, 117498-62-1; **38c**, amine derivative, 117498-68-7; **38c**, diamine derivative, 117498-69-8; **39**, 5271-67-0; **40a**, 56824-66-9; **40a**, 2-methoxy-4-methyl isomer, 117498-60-9; **40b**, 56824-67-0; **40b**, de-*O*-methyl derivative, 38281-77-5; **40b**, de-*O*-methyl nitro derivative, 117498-63-2; **42a**, 23806-24-8; **42b**, 14282-78-1; **43a**, 61341-26-2; **43b**, 32990-47-9; **44a**, 117498-42-7; **44b**, 117498-70-1; **45a**, 117498-43-8; **45b**, 117498-71-2; **46**, 554-14-3; **47**, 117498-44-9; **48a**, 117498-45-0; **48a**, diamine derivative, 117498-73-4; **48b**, 117498-72-3; **48b**, diamine derivative, 66938-94-1; **48c**, 66938-75-8; **48c**·HCl, diamine derivative, 117498-74-5; **49a**, 72080-83-2; **49b**, 57260-73-8; **49c**, 68076-36-8; **50a**, 117498-46-1; **50a**, diamine derivative, 117498-77-8; **50b**, 117498-75-6; **50b**, diamine derivative, 117498-78-9; **50c**, 117498-76-7; **50c**, diamine derivative, 117498-79-0; **51a**, 117526-27-9; **51b**, 117498-80-3; **52**, 13781-53-8; **53**, 117498-47-2; **53**, diamine derivative, 117498-81-4; **54**, 117498-48-3; **54**, acid chloride, 117498-90-5; **54**, methyl ester, 117498-82-5; **54**, methyl ester, diamine derivative, 117498-83-6; **55a**, 6964-21-2; **55b**, 37784-63-7; **56a**, 117498-49-4; **56a**, acid chloride, 117498-84-7; **56b**, 117498-87-0; **56b**, amine derivative, 117498-88-1; **56b**, diamine derivative, 117498-89-2; **57**, 117498-50-7; **57**, amine derivative, 117498-85-8; **57**, diamine derivative, 117498-86-9; **58**, 117498-51-8; **58**·HCl, deprotected derivative, 117498-93-8; **58**, diamine derivative, 117498-91-6; **59**, 117498-52-9; **59**, diamine derivative, 117498-94-9; **60**, 117498-53-0; **60**, amine derivative, 117498-95-0; **61**, 117498-54-1; **61**, diamine derivative, 117498-96-1; **62**, 117498-55-2; **62**, diamine derivative, 117498-98-3; **63**, 117498-56-3; **63**, diamine derivative, 117498-99-4; **64**, 111690-63-2; **64**, amine derivative, 117499-00-0; **64**, diamine derivative, 117499-01-1; $CH_3O_2CNHC(SCH_3)NH$, 39259-32-0; $CH_3O_2CNHC(SCH_3)NCO_2CH_3$, 34840-23-8; anisole, 100-66-3; 4-chloro-3-nitrobenzoic acid, 96-99-1; 4-chloro-3-nitrobenzoyl chloride, 38818-50-7; 3,4-dinitrobenzoic acid, 528-45-0; 3,4-dinitrobenzoyl chloride, 24376-18-9; 2-methylanisole, 578-58-5; 3-methylanisole, 100-84-5; 2-methyl-2-thiopseudourea sulfate, 867-44-7; thiophene-2-carboxylic acid, 527-72-0.