(2.5 L) and 1 M NH_4HCO_3 (2.5 L); fraction, 20 mL/5 min. Salt **5b** (0.448 g, 1.18 mmol, 60%) was isolated by evaporation of fractions 89–101. EI-MS, *m/e* (relative intensity): 505 (M^+ + 2 TMS (10)), 490 (M^+ + 2 TMS − 15 (100)), 577 (M^+ + 3 TMS (5)), 562 (M^+ + 3 TMS − 15 (22)). Relevant ^1H NMR data (D_2O): δ 7.57 (s, 1 H, H6), 6.05 (s, 1 H, H1′), 1.11 (t, 3 H, $CH_3(CH_2)_3$).

5-*n*-Pentylcytidine 3′,5′-Cyclic Monophosphate Ammonium Salt (6b). A 2.80-mmol (1.20-g) quantity of **6a** was used. Chromatographic conditions: **6b** was chromatographed twice; column, 4.7 × 90 cm; gradient, water (2.5 L) and 1 M NH_4HCO_3 (2.5 L); fractions, 20 mL/5 min. Compound **6b** (0.550 g, 1.40 mmol, 50%) was isolated by evaporation of fractions 165–178. EI-MS, *m/e* (relative intensity): 519 (M^+ + 2 TMS (≈8)), 504 (M^+ + 2 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.42 (s, 1 H, H6), 5.90 (s, 1 H, H1′), 0.91 (t, 3 H, $CH_3(CH_2)_4$).

5-*n*-Hexylcytidine 3′,5′-Cyclic Monophosphate Ammonium Salt (7b). A 1.22-mmol (0.540-g) amount of **7a** was used. Chromatographic conditions: **7b** was chromatographed twice; column, 4.7 × 90 cm; gradient, water (2.5 L) and 1 M NH_4HCO_3 (2.5 L); fractions 20 mL/5 min. Salt **7b** (0.222 g, 0.546 mmol, 46%) was isolated by evaporation of fractions 210–227. EI-MS, *m/e* (relative intensity): 533 (M^+ + 2 TMS (7)), 518 (M^+ + 2 TMS − 15 (100)), 605 (M^+ + 3 TMS (2)), 590 (M^+ + 3 TMS − 15 (13)). Relevant ^1H NMR data (DMSO-d_6/$CDCl_3$): δ 7.34 (s, 1 H, H6), 5.75 (s, 1 H, H1′), 0.88 (br t, 3 H, $CH_3(CH_2)_5$).

5-*n*-Octylcytidine 3′,5′-Cyclic Monophosphate Ammonium Salt (8b). A 1.00-mmol (0.469-g) amount of **8a** was used. Chromatographic conditions: **8b** was chromatographed twice; column, 4.7 × 90 cm; gradient, water (2.5 L) and 1.5 M NH_4HCO_3 (2.5 L); fractions 20 mL/5 min. Compound **8b** (0.193 g, 0.444 mmol, 44%) was isolated by evaporation of fractions 207–225. EI-MS, *m/e* (relative intensity): 561 (M^+ + 2 TMS (≈10)), 546 (M^+ + 2 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.49 (s, 1 H, H6), 5.88 (s, 1 H, H1′), 0.91 (br t, 3 H, $CH_3(CH_2)_7$).

Cytostatic Assays. L1210/0 and L1210/araC cell lines were characterized as described.[27] The cytostatic assays were performed according to previously established procedures.[32]

Antiviral assays were performed as reported previously.[33] The origin and preparation of the virus stocks have also been documented in ref 31.

Acknowledgment. Support of this work by grants from the National Cancer Institute of the Public Health Service (CA 11045, to W.G.B.) and the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (to E.D.C.) is gratefully acknowledged. We thank Dr. Robert Suva, Sophia Mah, James P. FitzGerald, and Dr. Jon P. Miller, Stanford Research Institute International, for the protein kinase data, and Lizette Van Berckelaer and Anita Van Lierde for excellent technical assistance.

Registry No. **1a**, 117309-80-5; **1b**, 117309-87-2; **2a**, 117309-81-6; **2b**, 117309-88-3; **3a**, 117309-82-7; **3b**, 117309-89-4; **4a**, 117309-83-8; **4b**, 117309-90-7; **5a**, 117340-75-7; **5b**, 117309-91-8; **6a**, 117309-84-9; **6b**, 117309-92-9; **7a**, 117309-85-0; **7b**, 117309-93-0; **8a**, 117309-86-1; **8b**, 117309-94-1; 5-methylcytidine hydrochloride, 117309-75-8; 5-ethylcytidine hydrochloride, 34210-56-5; 5-isopropylcytidine hydrochloride, 117309-76-9; 5-*n*-propylcytidine, 66270-32-4; 5-*n*-butylcytidine hydrochloride, 117309-77-0; 5-*n*-pentylcytidine hydrochloride, 117309-78-1; 5-*n*-hexylcytidine, 90012-90-1; 5-*n*-octylcytidine, 117309-79-2.

Supplementary Material Available: Tables listing ^{13}C NMR data for **1a–8a** and **1b–8b** (2 pages). Ordering information is given on any current masthead page.

(32) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Mol. Pharmacol.* **1981**, *19*, 321.
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Synthesis of Acylguanidine Analogues: Inhibitors of ADP-Induced Platelet Aggregation

Edward W. Thomas,* Edward E. Nishizawa, David C. Zimmermann, and Davey J. Williams

The Upjohn Company, Kalamazoo, Michigan 49001. *Received April 4, 1988*

Routine screening of compounds for inhibition of ADP-induced platelet aggregation in vitro revealed that 1,1′-hexamethylenebis[3-cyclohexyl-3-[(cyclohexylimino)(4-morpholinyl)methyl]urea] (1) was active and represented the first example of a bis(acylguanidine) with possible antithrombotic activity. In order to develop a structure–activity relationship for this class of compounds, we synthesized a number of new bis(acylguanidines). These were tested in vitro, and several analogues were also active. Ex vivo testing revealed that compounds **22**, **41**, **58**, and **70–73** were orally active in rats or guinea pigs.

Researchers continue to emphasize the role of platelet-active drugs in the control of cardiovascular and cerebrovascular diseases.[1] These agents are believed to control or ameliorate the biochemical events that lead to thrombosis and vessel-wall damage. The common explanation for the series of events leading to thrombosis is fracture of the vessel's endothelium layer followed by platelet adherence to the exposed subendothelium. The adhering platelets then release other agents including thromboxane A_2 and adenosine diphosphate (ADP). These agents are responsible for the growth of platelet aggregates. At the same time, platelet membrane changes encourage the formation of thrombin. This induces further platelet aggregation and enmeshes the platelet mass in fibrin, stabilizing a thrombus.[2] In previous work on platelet drugs, we developed a compound that inhibits platelet aggregation induced by collagen.[3] Subsequently, we focused on an agent that would be a well-tolerated, orally active inhibitor of ADP-induced platelet aggregation. Others have also identified inhibitors of primary-wave ADP-induced platelet aggregation.[4]

During the course of our work, we discovered compound 1, which inhibits primary-wave ADP-induced platelet aggregation in human platelet-rich plasma (PRP), in vitro. This molecule presented an attractive lead as it possesses

(1) (a) de Gaetano, G.; Cerletti, C.; Dejana, E.; Vermylen, J. *Drugs* **1986**, *31*, 517–549. (b) Weiss, H. J. *Platelets: Pathophysiology and Antiplatelet Drug Therapy*; Alan R. Liss: New York, 1983.

(2) (a) Packham, M. A.; Mustard, J. F. *Semin. Hematol.* **1986**, *23*, 8–21. (b) Born, G. V. R.; Gorog, P.; Begent, N. A. *Am. J. Med.* **1983**, *74*(6a), 2–9. (c) Gallus, A. S. *Med. J. Aust.* **1985**, *142*, 41–47.

(3) Thomas, E. W.; Nishizawa, E. E.; Zimmermann, D. C.; Williams, D. J. *J. Med. Chem.* **1985**, *28*, 442–446.

(4) (a) Whittle, B. J. R.; Moncada, S. *Circulation* **1985**, *72*, 1219–1225. (b) De Gaetano, G.; Bertele, V. *Agents Actions* **1984**, *14*, 109–112.

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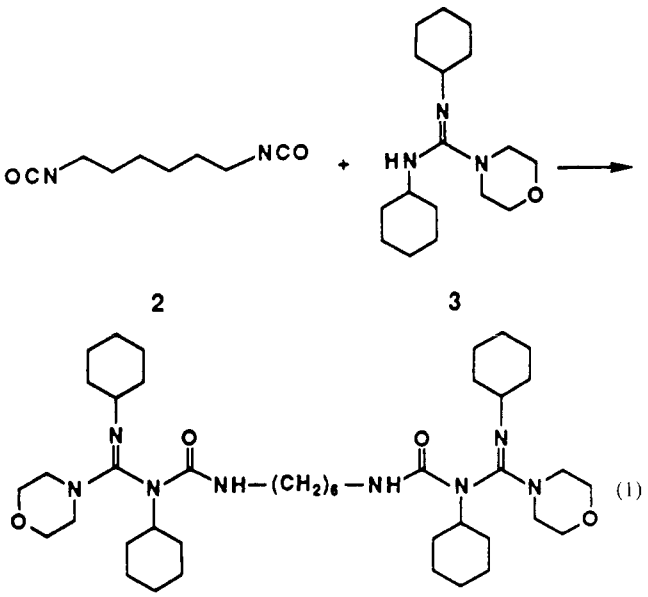
an array of functional groups: urea, guanidine, and alkyl diamine. One could envision a number of novel analogues, evolving from a series of functional-group manipulations and structural modifications of **1**, which could be tested in vitro by the method of Born and Cross.[5] A common feature shared by this compound with other platelet inhibitory drugs, dipyridamole[1a] and anagrelide,[6] is the guanidine unit. We report herein the systematic investigation of the structure–activity relationship (SAR) of **1**, which culminated in the discovery of compounds **22**, **41**, **58**, and **70–73**, which are orally active inhibitors of ADP-induced platelet aggregation.

Although compound **1** contains several functional groups, this molecule is readily synthesized, in CH_2Cl_2 at room temperature from two commercially available starting materials, diisocyanate **2** and guanidine **3**.[7] This compound was isolated as a mixture of geometrical isomers, as evidenced by several spots on TLC and by twinned peaks in the ^{13}C NMR spectrum. Likewise, for all subsequent analogues, no attempt was made to separate the guanidine isomers. Employing this chemistry, one need only vary the diisocyanate and guanidine starting materials to create an array of analogues.



2 3

(1)

1

The first analogues synthesized were ones in which the guanidine portion of the molecule was modified.[8] Following a literature method for the synthesis of guanidines,[8c] we treated commercially available carbodiimides with excess amine, at reflux, to afford guanidines **4–14**, Table I. Dicyclohexylcarbodiimide (DCC) did not react with excess dicyclohexylamine even after 24 h at reflux. However, lithium dicyclohexylamide reacted readily with DCC to form guanidine **15**. In a similar manner, guanidines **16–18** were formed. Guanidines **19** and **20** were derived from lithium dimethylamide and the corresponding carbodiimides.

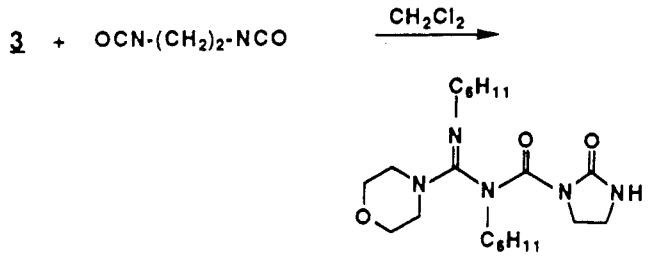
All the guanidines in Table I were treated with diisocyanate **2** to form the analogues found in Table II. Unlike **3**, not all of the guanidines in Table I reacted smoothly with a diisocyanate in methylene chloride at room temperature. Guanidine **12** failed to react with **2** even after 24 h at reflux in methylene chloride. However, when the more polar solvent acetonitrile was substituted for methylene chloride, **12** reacted with **2** at room temperature to yield **28**, and **19** reacted with **2** to form **24**, which crystallized out as analytically pure material. On the basis of these observations, acetonitrile was employed as the reaction solvent in all subsequent cases where the guanidine was derived from either dicyclohexylcarbodiimide or di-*p*-tolylcarbodiimide. Methylene chloride or acetonitrile was found to be a suitable solvent for reactions involving guanidines derived from diisopropylcarbodiimide. As can be seen from Table II, we were unable to prepare analogues **27** and **37**. Conducting the reactions in either acetonitrile or methylene chloride afforded only polymer. The reasons for these results are not clear. In vitro screening of all the compounds in Table II revealed that none of the analogues were as active as the lead, **1**. Therefore, the guanidine portion of **1**, derived from **3**, was deemed necessary for activity and was retained in all subsequent analogues.

To explore the role of the alkyl chain's length toward biological activity, we synthesized a series of homologues of **1**. The diisocyanates needed for the study were prepared in higher yield by a slight modification of literature methods,[9] in which the reaction solvent chlorobenzene was substituted for *o*-dichlorobenzene. The various diisocyanates were then treated with guanidine **3**, in methylene chloride, to form the analogues in Table III. The diisocyanate where $n = 2$, prepared by literature methods,[10] did not form compound **38** as desired; instead, imidazolidone **46** was isolated.[11] In an attempt to thwart the undesired intramolecular reaction, ethylene diisocyanate was added to a cooled (−10 °C) methylene chloride solution saturated with a 2-fold excess of guanidine **3**. The only product isolated was **46** in 50% yield. Likewise, the diisocyanate where $n = 3$ afforded as the main product the tetrahydropyrimidinone **47** in 51% yield. Clearly, **46** and **47** are the major compounds because the intramolecular reaction is favored entropically due to the formation of a five- and six-membered ring.

Listed in Table III are compounds **40–44**, which were active and nearly equipotent, while **45** was inactive, vide infra. Due to the apparent lack of an ideal chain length, the role of the chain itself was questioned. Guanidine **3** and butyl isocyanate afforded analogue **48**, which was inactive.

Clearly, the alkyl chain must serve to link the guanidine units such that they are oriented in a fixed relationship to each other, where they then impart their biological activity.[12] We next focused on how the chain's flexibility influenced the spatial relationship of the guanidines. Diisocyanates **51–53** were available commercially, and

(5) Born, G. V. R.; Cross, M. J. *J. Physiol.* **1963**, *168*, 178–195.
(6) Fleming, J. S.; Buyniski, J. P. *New Drugs Annu.: Cardiovasc. Drugs* **1983**, *1*, 277–294.
(7) Others have reacted isocyanates with guanidines: Ulrich, H. *Acc. Chem. Res.* **1969**, *2*, 189.
(8) Guanidines have been prepared by a number of methods: (a) Barton, D. H. R.; Elliott, J. D.; Gero, S. D. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2085–2090. (b) Palomo, C.; Mestres, R. *Synthesis* **1980**, 755–757. (c) Ger. Pat. 2545648; *Chem. Abstr.* **1977**, *87*, 68158j. (d) U.S. Pat. 3961056; *Chem. Abstr.* **1976**, *85*, 83235j. (e) Verheyden, J. P. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1964**, *86*, 1236–1241. (f) Ger. Pat. 2716477; *Chem. Abstr.* **1979**, *90*, 38552c.
(9) Siefkin, W. *Justus Liebigs Ann. Chem.* **1949**, *562*, 75–136.
(10) Sayigh, A. A. R.; Tilley, J. N.; Ulirch, H. *J. Org. Chem.* **1964**, *29*, 3344–3346.
(11) Sayigh, A. A. R.; Tilley, J. N. *J. Org. Chem.* **1964**, *29*, 3347–3350.
(12) The spatial relationship of amines in symmetrical molecules has been evaluated as to its role in inhibiting platelet aggregation: Quintana, R. P.; Lasslo, A.; Dugdale, M.; Goodin, L. L. *Thromb. Res.* **1981**, *22*, 665–680.



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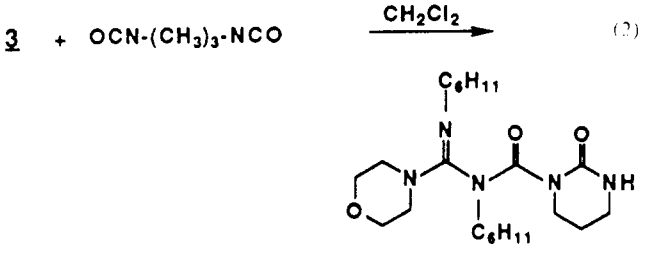

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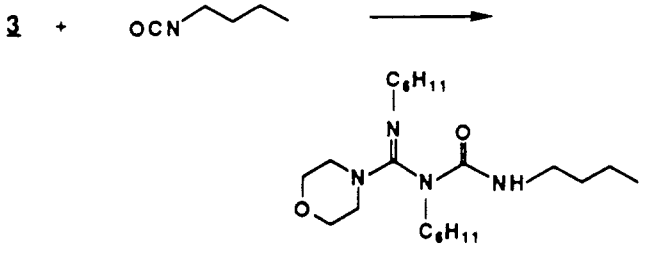
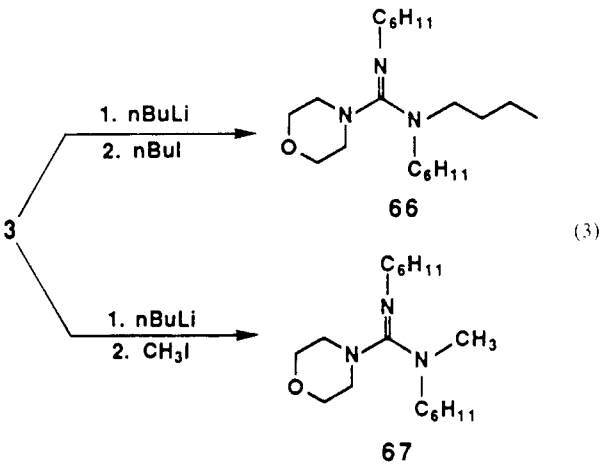


48

diisocyanates **49**, **50**, and triisocyanate **54** were prepared by standard literature methods.[13] The diisocyanates and compound **3**, in CH_2Cl_2 at room temperature, afforded the analogues in Table IV. The benzyl ureas **58–60** were found to be more stable than the aryl ureas **55–57**, which 1 day after purification exhibited a new spot on TLC. Only analogues **58** and **60** were active. This illustrates that the chain must be long enough and flexible enough that the guanidines can approach each other in space. The corresponding guanidines in compounds **56**, **57**, and **59** cannot approach each other due to para substitution of the aromatic ring. Even though in analogue **55** the guanidines are separated by only five carbons, the system may not retain biological activity due to loss in flexibility of the chain.

The structure–activity relationship of analogues became focused, as constraints on the alkyl chain and the guanidine moiety were found. To assess whether the urea portion of the molecule was necessary, analogues **61–64** in Table V were synthesized from biselectrophiles and guanidine **3** in CH_2Cl_2 at room temperature. Lacking literature precedent for the alkylation of tetrasubstituted guanidines,[8] we laid the groundwork for the preparation of **65** by preparing **66** and **67**. Lithiation of **3** with *n*-butyllithium, followed by reaction with methyl iodide and *n*-butyl iodide, afforded alkylated compounds **67** and **66**, respectively, providing another method for the preparation of hindered guanidines.[8a] The alkylation of lithiated **3** with 1,10-diiododecane proved more sluggish, affording only a 17% yield of **65**, Table V. None of these analogues were active.



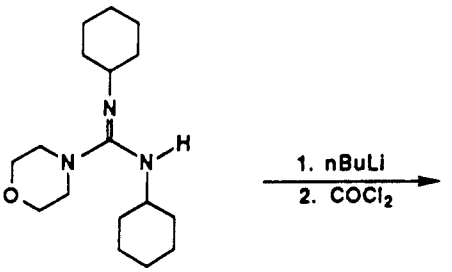
66

67

(3)

Compound **1**, although a potent in vitro inhibitor of ADP-induced platelet aggregation, exhibited no oral activity, Table IX. Any orally active compound must first survive stomach acid and enzymes which have the capacity to destroy some drugs before they are absorbed.[14] Since none of the analogues prepared were more potent in vitro than **1**, the decision was made to focus on modifications of **1** which may impart oral activity.

Treatment of **1** in MeOH with HCl gas formed the bis(hydrochloride salt) **68**. The carbamoyl chloride **69** (IR 1740 cm^{-1}), prepared from lithiated **3** and phosgene, decomposed within 4 h, and attempts to purify the material by chromatography or distillation failed. The crude carbamoyl chloride and corresponding secondary amines afforded N-substituted urea analogues, Table VI. These analogues are more hindered around the urea linkage, in analogy to peptides which when substituted on the nitrogen are more stable toward enzymatic cleavage.[15] Likewise the N-substituted urea **71** was less polar than unsubstituted **1** as judged by mobility on TLC.



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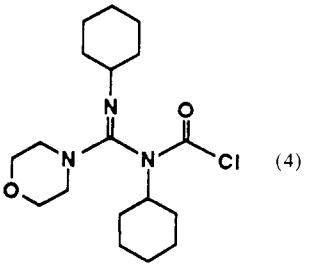

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(4)

(13) Ulrich, H. *Condensation Monomers*; Wiley: New York, 1972; p 370–463.

(14) Katzung, B. G. In *Basic and Clinical Pharmacology*, 3rd ed.; Katzung, B. G., Ed.; Appleton and Lange: Norwalk, 1987; Chapter 1.

(15) Wiedhup, K. In *Topics in Pharmaceutical Sciences*; Briemer, D., Speiser, P., Eds.; Elsevier/North-Holland Biomedical: Amsterdam, 1981; pp 307–324.

Table I. Synthesis of Tetrasubstituted Guanidines

$$RN{=}C{=}NR + R_1H \rightarrow RN{=}C(R_1)NHR$$

compd	R	R_1	yield, %	compd	R	R_1	yield, %
4	C_6H_{11}		92[a]	13	p-$CH_3C_6H_4$		70
5	C_6H_{11}		73[a]	14	p-$CH_3C_6H_4$		96[a]
6	C_6H_{11}		56[a]	15	C_6H_{11}	$N(C_6H_{11})_2$	59[c]
7	$(CH_3)_2CH$		83	16	$(CH_3)_2CH$	$N(C_6H_{11})_2$	69
8	$(CH_3)_2CH$		50	17	C_6H_{11}	$N(C_6H_5)_2$	59
9	$(CH_3)_2CH$		72	18	$(CH_3)_2CH$	$N(C_6H_5)_2$	66
10	$(CH_3)_2CH$		80	19	C_6H_{11}	$N(CH_3)_2$	67[a]
11	p-$CH_3C_6H_4$		68	20	$(CH_3)_2CH$	$N(CH_3)_2$	67[b]
12	p-$CH_3C_6H_4$		74				

[a] The salts of these guanidines have been prepared previously; see ref 8d. [b] See ref 8f. [c] Isolated as the hydrochloride salt.

Table II. Variation of the Guanidine Portion of Compound 1

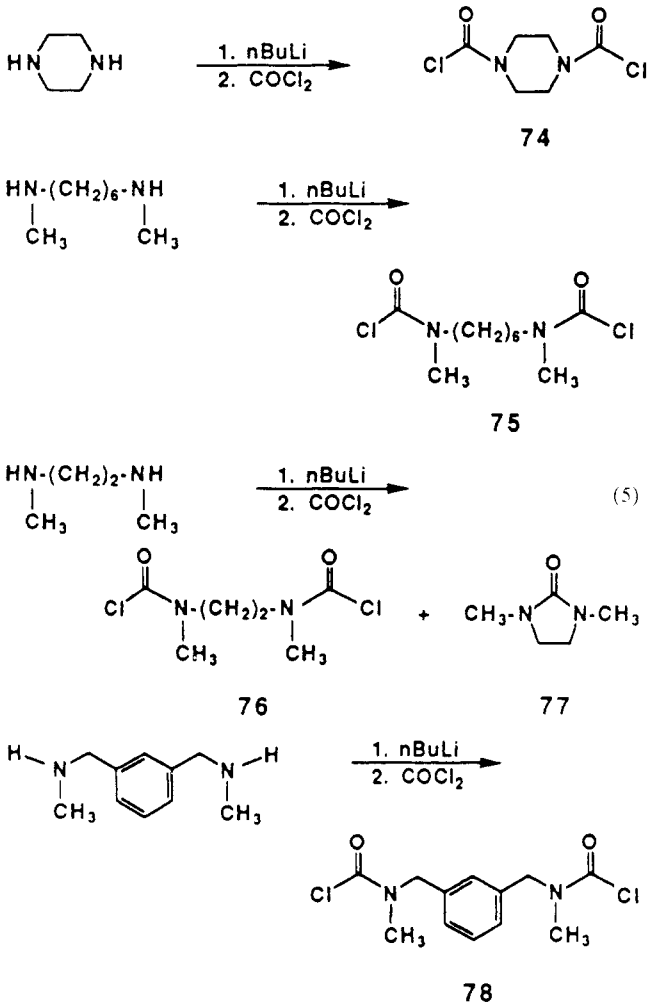
$$RN{=}C(R_1)NHR + OCN(CH_2)_6NCO \rightarrow RN{=}C(R_1)N(R)C(O)NH(CH_2)_6NHC(O)N(R)C(R_1){=}NR$$

compd	R	R_1	solvent	yield, %	compd	R	R_1	solvent	yield, %
1	C_6H_{11}		CH_2Cl_2	70	29	p-$CH_3C_6H_4$		CH_3CN	87
21	C_6H_{11}		CH_3CN	75	30	p-$CH_3C_6H_4$		CH_3CN	64
22	C_6H_{11}		CH_3CN	55	31	$(CH_3)_2CH$		CH_3CN	36
23	C_6H_{11}		CH_3CN	73	32	$(CH_3)_2CH$		CH_2Cl_2	61
24	C_6H_{11}	$N(C_6H_{11})_2$	CH_3CN	83	33	$(CH_3)_2CH$		CH_2Cl_2	51
25	C_6H_{11}	$N(C_6H_{11})_2$	CH_3CN/CH_2Cl_2	57	34	$(CH_3)_2CH$		CH_3CN	34
26	C_6H_{11}	$N(C_6H_5)_2$	CH_3CN	79	35	$(CH_3)_2CH$	$N(CH_3)_2$	CH_2Cl_2	83
27	p-$CH_3C_6H_4$		CH_3CN or CH_2Cl_2	0	36	$(CH_3)_2CH$	$N(CH_3)_2$	CH_2Cl_2	26
28	p-$CH_3C_6H_4$		CH_3CN	65	37	$(CH_3)_2CH$	$N(C_6H_5)_2$	CH_3CN or CH_2Cl_2	0

All the compounds in Table VI exhibited activity in vitro. In order to generate sufficient material for oral dosing, we devised a higher yielding synthesis of these analogues in which diamines were acylated and then reacted with **3**. The bis(carbamoyl chlorides) of both piperazine, **74**, and *N*,*N*′-dimethylhexanediamine, **75**, were prepared according to published methods by treatment of the diamine with phosgene in refluxing chlorobenzene.[16] It was found that bis(carbamoyl chlorides) could also be prepared by addition of the corresponding lithium diamine dianion to phosgene at low temperature. This procedure allowed for shorter reaction times and better yields of the white solids **74** and **75**, which after 12 months were homogeneous as shown by TLC.

Following similar chemistry, we isolated the bis(carbamoyl chloride) **76** in 47% yield and the expected imidazolidinone **77** in 30% yield. Compound **78** was also produced in 59% yield by this general method. The bis(carbamoyl chloride) **75** was treated with **3** (4 equiv), and after 24 h at room temperature, none of the desired product could be detected by TLC.[17] Addition of the

(16) (a) Schindler, N.; Ploger, N. *Chem. Ber.* **1971**, *104*, 969–971. (b) Hasserodt, U. *Chem. Ber.* **1968**, *101*, 113–120.



lithium anion of **3** to bis(carbamoyl chloride) **75** (1 equiv) led to **71** in 66% yield. Other analogues were prepared in a similar manner, Table VII.

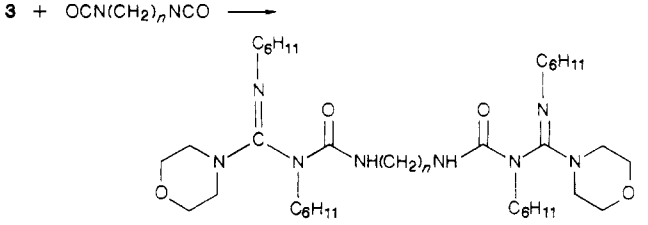
Biological Results and SAR Summary

All guanidines and analogues of **1** were screened for inhibition of ADP-induced platelet aggregation in human platelet-rich plasma by the method of Born and Cross.[5] The advantage of this screen is the ability to easily test a large number of compounds and thereby develop a large data base to formulate a structure–activity relationship (SAR). Active analogues are listed in Table VIII in decreasing order of activity.

From the SAR study, the only in vitro active compounds were those possessing linked acylguanidines. Those analogues with chain lengths from C-4 to C-9, **40–44** and **1**, were active but were not equally potent, as compound **1** remained the most potent in this series. Meta-substituted analogue **58** was active, while para-substituted derivatives **56** and **57** were inactive. This suggested that a specific orientation of the two guanidine units is important for inhibition of the active site responsible for platelet aggregation. The proper selection of the guanidine unit also proved important as only one analogue, **22**, with a guanidine unit different from that found in **1** exhibited activity, but it was less active than **1**. Substitution on the nitrogen also maintained activity, although **70**, which is more constrained than **71–73**, was less active than the others.

(17) Although amines have been added to carbamoyl chlorides, to our knowledge guanidines had not been added: Rivett, D. E.; Wilshire, J. F. K. *Aust. J. Chem.* **1966**, *19*, 869–875.

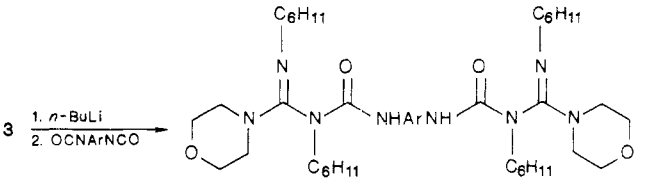
Table III. Synthesis of Alkyl Chain Analogues

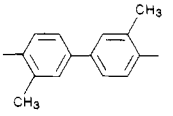
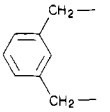
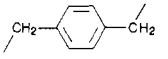
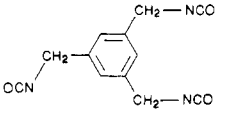


compd	*n*	yield,[a] %
38	2	0
39	3	0
40	4	39
41	5	64
42	7	60
43	8	59
44	9	44
45	10	69

[a] All the reactions were run in CH_2Cl_2.

Table IV. Synthesis of Rigid Tether Analogues

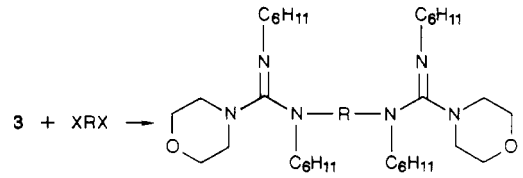


diisocyanate compd	Ar	urea compd	yield, %
49		**55**	21
50		**56**	13
51		**57**	49
52		**58**	65
53		**59**	41
54		**60**	29

The active compounds in Table VIII were tested for stability in whole human blood. Those compounds that retained their ability to inhibit ADP-induced platelet aggregation activity were tested for oral activity in rats and guinea pigs. The results from the oral studies are listed in Table IX.

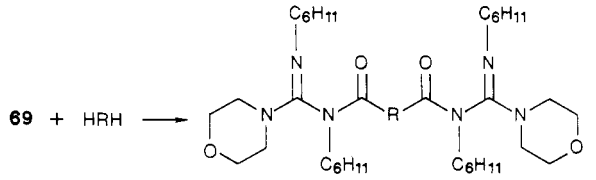
Compound **1**, its bis(hydrochloride salt) **68**, and chain-length homologues **40** and **42** were not orally active in rats. However, the chain-length homologue **41** did possess oral activity in rats. Unlike the in vitro activity in human plateletes, there does seem to be an optimum chain length (five carbons) for oral activity in rats. N-substitution of the urea nitrogen did impart ex vivo activity to **70**, **71**, and **73** in the rat. Yet, N-substitution of the nitrogen did not guarantee activity as **72** was inactive in the rat. Compound **22**, possessing the piperidine-derived guanidine, also ex-

Table V. Analogues Devoid of the Urea Group



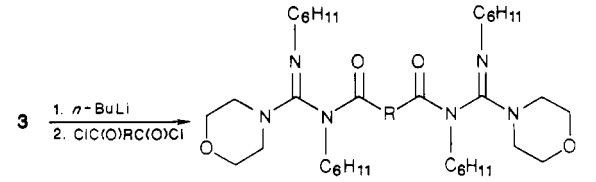
compd	X	R	yield, %
61	Cl	$C(O)(CH_2)_6C(O)$	81
62	Cl	$C(O)(CH_2)_8C(O)$	65
63	Cl	C(O)C(O)	77
64	Cl	$C(O)O(CH_2)_6OC(O)$	41
65	I	$(CH_2)_{10}$	17

Table VI. Synthesis of N-Substituted Urea Analogues



compd	R	yield, %
70		9
71	$N(CH_3)(CH_2)_6N(CH_3)$	3
72	$N(CH_3)(CH_2)_2N(CH_3)$	7

Table VII. Synthesis of N-Substituted Urea Analogues by Improved Route



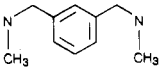
compd	R	yield, %
70		30
71	$N(CH_3)(CH_2)_6N(CH_3)$	66
72	$N(CH_3)(CH_2)_2N(CH_3)$	74
73		62

Table VIII. In Vitro ADP-Induced Platelet Inhibitory Activity of Guanidine Analogues

compd	IC_{50},[a] μg/mL	rel potency[b]	incubn[c]
1	3.2	0.003	+
58	3.2	0.003	+
71	3.2	0.003	+
72	3.2	0.003	+
73	3.2	0.003	+
22	10	0.001	+
40	10	0.001	+
41	10	0.001	+
42	10	0.001	+
43	10	0.001	-
44	10	0.001	-
60	10	0.001	-
70	10	0.001	+
68	10	0.001	+
PGE_1	0.01	1	

[a] Concentration of analogue required to inhibit, by 50% (IC_{50}), ADP-induced platelet aggregation in human PRP. [b] Relative to PGE_1. [c] Activity following incubation for 30 min in human whole blood; +, active; -, inactive.

hibited ex vivo activity in the rat. The structure–activity relationship of compounds tested ex vivo in the rat is complex. By relating oral activity to lipophilicity, one finds that compounds **22**, **70**, **72**, and **73** are orally active in rats and less polar than compound **1**. Yet, compound **41** is orally active in rats and is similar in polarity to compound **1**. N-Alkyl substitution was not necessary to avoid possible enzymatic cleavage as compounds **22** and **58** were orally active in rats.

The species variability in this series also added to the complexity of developing a clear SAR picture. One can contrast analogue **72**, which was active only in guinea pigs, to its chain-length homologue **71**, which was active only in rats.

All of these compounds exhibited gastrointestinal side effects which would limit their use as therapeutic agents. In the stomachs of the sacrificed animals, fluid had accumulated and evidence for lack of stomach emptying was observed. The drugs were active at high doses (100 mg/kg), and the inactivity at lower doses may be due to impaired stomach motility. Whether the side effects can be eliminated for this class of compounds is not known at this time.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer 297 spectrometer. ^1H NMR spectra were recorded on a Varian Associates EM-390 (90 MHz) spectrometer and are reported in δ units from internal tetramethylsilane. ^{13}C NMR were recorded on a Varian CFT-20 spectrometer and are reported in parts per million from tetramethylsilane on the δ scale. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. Mass spectra were recorded on a Varian MAT-CH5 spectrometer. Combustion analyses were performed by the Upjohn Physical and Analytical Chemistry Laboratory and by the Spang Microanalytical Laboratory. Unless specified, all solvents and reagents were used without further purification. THF was dried over molecular sieves.[18]

Synthesis of Tetrasubstituted Guanidines. In Table I, guanidines **4–14** were prepared by method A and guanidines **15–20** were prepared by method B. The novel guanidines are listed in Table X with their isolation and characterization data, as compounds **4–6**, **14**, **19**,[8d] and **20**[8f] have been synthesized previously.

Method A for the Preparation of *N*,*N*′-Dicyclohexyl-1-pyrrolidinecarboxamidine (4). Dicyclohexylcarbodiimide (3.0 g, 14.8 mmol) and pyrrolidine (10 mL, 120 mmol) were heated at reflux for 1 h. The excess pyrrolidine was removed in vacuo, and the last traces of pyrrolidine were removed at 0.1 mm to give **4** (3.78 g, 92%), as a colorless oil: ^1H NMR ($CDCl_3$) δ 0.90 (m, 24 H), 2.80–3.15 (m, 2 H), 3.20–3.45 (m, 4 H), 7.42 (s, 1 H).

Method B for the Preparation of *N*′,*N*″-Dicyclohexyl-*N*,*N*-dimethylguanidine (19). *n*-Butyllithium (23.5 mL, 40.0 mmol) was added dropwise to a cooled solution (−78 °C) of dimethylamine (6 mL, 91 mmol) in THF (100 mL). The reaction mixture was warmed to −20 °C over 1 h and was then recooled to −78 °C. Dicyclohexylcarbodiimide (8.25 g, 40.0 mmol) dissolved in THF (25 mL) was added, and the reaction was continued overnight and the mixture allowed to warm to room temperature. The reaction was quenched with chilled H_2O (30 mL). The organic portion was dried and concentrated in vacuo to an oil with some crystals present. The oil was dissolved in hexane and filtered to remove the solid. The hexane was concentrated in vacuo to an oil, which was bulb-to-bulb distilled in a Kugelrohr apparatus to yield **19** (7.8 g, 67%), as a colorless oil: bp 80 °C at 0.1 mm; ^1H NMR ($CDCl_3$) δ 0.90–2.05 (m, 20 H), 2.65 (s, 6 H), 2.70–3.15 (m, 2 H), 7.40 (s, 1 H).

The analogues found in Tables II–IV and compounds **46–48** and **61–64** were synthesized under similar conditions except for solvent. One specific procedure follows. The remaining analogues

(18) Burfield, D. R.; Gan, G.; Smithers, R. H. *J. Appl. Chem. Biotechnol.* **1978**, *22*, 23–30.

Table IX. Ex Vivo Testing of U-40,600 Analogues in Rat and/or Guinea Pig

compd	species	dose, mg/kg	n	extent of aggregation		p value
				control	treated[a]	
1	rat	100	4	77.5 ± 7.8	80.8 ± 3.6	0.693
71	rat	100	5	80.0 ± 14.7	52.0 ± 13.5	0.001
		30	5	80.0 ± 14.7	81.2 ± 20.9	0.872
	guinea pig	100	5	53.0 ± 9.0	41.0 ± 7.0	0.115
72	rat	100	4	84.0 ± 2.3	100+	0.062
	guinea pig	100	4	67.0 ± 8.4	0	0.000
		30	4	77.0 ± 9.7	62.7 ± 20.9	0.070
22	rat	100	4	92.8 ± 11.3	46.2 ± 8.9	0.000
		50	4	92.8 ± 11.3	86.0 ± 9.3	0.379
		25	4	92.8 ± 11.3	83.2 ± 2.5	0.217
	guinea pig	100	3	66.3 ± 8.8	48.7 ± 21.3	0.147
		50		50.7 ± 14.6	47.7 ± 22.3	0.801
58	guinea pig	50	3	63.7 ± 19.1	19.3 ± 13.0	0.001
68	rat	100	4	80.5 ± 6.4	84.5 ± 13.8	0.698
42	rat	100	4	69.5 ± 3.3	70.5 ± 8.2	0.922
40	rat	100	4	69.5 ± 3.3	73.5 ± 10.3	0.878
41	rat	100	4	72.8 ± 9.2	41.0 ± 9.5	0.007
70	rat	100	4	72.8 ± 9.2	44.8 ± 11.7	0.002
73	rat	100	4	85.5 ± 1.2	38.5 ± 13.3	0.000

[a] After 2 h.

Table X. Physical and Analytical Data for Tetrasubstituted Guanidines

no.	formula	NMR ($CDCl_3$) δ	anal.	mp, °C/bp, °C (mm)	isolation[a,b,c]
7	$C_{11}H_{23}N_3$	1.10 (d, 12 H), 1.68–1.89 (m, 4 H), 3.14–3.59 (m, 6 H), 7.40 (s, 1 H)	CHN	56–58 (0.03)	distilled
8	$C_{12}H_{25}N_3$	1.10 (d, 12 H), 1.41–1.70 (m, 6 H), 2.91–3.19 (m, 4 H), 3.10–3.63 (m, 2 H), 7.41 (s, 1 H)	CHN	56 (0.1)	distilled
9	$C_{11}H_{23}N_3S$	1.05 (d, 6 H), 1.10 (d, 6 H), 2.51–2.73 (m, 4 H), 3.11–3.55 (m, 6 H), 7.37 (s, 1 H)	CHNS	95–97 (0.01)	distilled
10	$C_{11}H_{23}N_3O$	1.09 (d, 6 H), 1.15 (d, 6 H), 3.08 (t, 4 H), 3.20–3.61 (m, 2 H), 3.70 (t, 4 H), 7.42 (s, 1 H)	CHN	56–57/60.2 (0.2)	sublimed
11	$C_{19}H_{23}N_3$	1.76–1.97 (m, 4 H), 2.30 (s, 6 H), 3.24–3.52 (m, 4 H), 5.53 (br s, 1 H), 6.84 (d, 4 H), 7.13 (d, 4 H)	CHN	84–280	A
12	$C_{20}H_{25}N_3$	1.33–1.68 (m, 6 H), 2.24 (s, 6 H), 3.14–3.40 (m, 4 H), 5.37 (br s, 1 H), 6.83 (d, 2 H), 7.04 (d, 2 H)	CHN	56–67	A
13	$C_{19}H_{23}N_3S$	2.27 (s, 6 H), 2.47–2.70 (m, 4 H), 3.50–3.76 (m, 4 H), 4.82 (br s, 1 H), 6.83 (d, 4 H), 7.10 (d, 4 H)	CHNS	157–177	A + B
15	$C_{25}H_{45}N_3$·HCl	1.00–2.00 (m, 40 H), 2.78–3.45 (m, 3 H), 3.66–3.87 (m, 1 H), 7.40 (s, 1 H)	CHN	280–291	C
16	$C_{19}H_{37}N_3$	0.90–2.00 (m, 20 H), 1.08 (d, 12 H), 2.91 (br s, 2 H), 3.73 (br s, 2 H), 7.30 (s, 1 H)	CHN	125–126 (0.10)	distilled
17	$C_{25}H_{33}N_3$	0.95–1.85 (m, 20 H), 3.40 (br s, 1 H), 6.95–7.40 (m, 11 H)	CHN	–	D
18	$C_{19}H_{25}N_3$	0.98 (d, 12 H), 3.56–3.87 (m, 2 H), 6.96–7.45 (m, 10 H)	CHN	55–57/118–121 (0.15)	distilled

[a] Recrystallized from the following: A = hexane; B = benzene. [b] Isolated as the HCl salt and recrystallized from the following: C = CH_3CN/MeOH. [c] Chromatographed on SiO_2 and eluted with the following: D = Et_2O/NH_3.

synthesized this way are listed in Table XI along with isolation and characterization data.

1,1′-Hexamethylenebis[3-cyclohexyl-3-[(cyclohexylimino)(4-morpholinyl)methyl]urea] (1). Guanidine **3** (2.93 g, 10 mmol) was added to hexamethylene diisocyanate (840 mg, 5.0 mmol), which was dissolved in CH_2Cl_2 (15 mL) at room temperature. After 22 h, no isocyanate stretch was seen in the IR spectrum for an aliquot of the reaction mixture. The reaction mixture was concentrated in vacuo to 3.88 g of crude material. This was triturated with CH_3CN, affording pure **1** (3.32 g, 88%): mp 138–140 °C. The melting point of **1** was raised by a further trituration of 100 mg with CH_3CN to afford 80 mg of **1**: mp 144–146 °C; ^1H NMR ($CDCl_3$) δ 0.90–2.15 (m, 48 H), 3.00–3.45 (m, 14 H), 3.50–3.85 (m, 10 H), 4.40–4.90 (m, 2 H); ^{13}C NMR ($CDCl_3$) 155.0, 146.9, 66.6, 57.3, 56.1, 46.9, 40.3, 35.2, 34.7, 31.2, 31.1, 30.4, 26.6, 26.1, 26.0, 25.7, 24.7, 24.5 ppm. Anal. ($C_{42}H_{74}N_8O_4$) C, H, N.

The analogues found in Tables VI and VII and **65–67** were synthesized by protocol C or D. One specific procedure by each method follows. The remaining analogues synthesized by this method are listed in Table XII along with isolation and characterization data.

Method C for the Preparation of *N,N″*-(1,10-Decanediyl)bis(*N,N′*-dicyclohexyl-4-morpholinecarboximidamide) (65). Guanidine **3** (7.28 g, 24.8 mmol) was dissolved in THF (80 mL) and was cooled to −78 °C. *n*-Butyllithium (15.5 mL, 24.8 mmol, 1.6 M in hexane) was added to the reaction mixture, and the temperature was then kept at 0 °C for 1 h. Then, 1,10-diiododecane (3.26 g, 8.26 mmol) was added and the reaction was continued for 96 h at room temperature. Water (200 mL) was added to quench the reaction, and the organic layer was dried and concentrated in vacuo. The excess guanidine **3** was removed under high vacuum (140 °C at 0.4 mm), and the pot residue was chromatographed (200 g of SiO_2, CH_3OH/Et_3N, 15%) to afford solid product. The solid was recrystallized from CH_3CN to yield **65** (1.0 g, 17%): mp 103–107 °C; ^1H NMR ($CDCl_3$) δ 0.90–2.10 (m, 56 H), 2.45–3.30 (m, 16 H), 3.50–3.75 (m, 8 H). Anal. ($C_{44}H_{80}N_6O_2$) C, H, N.

Method D for the Synthesis of *N,N″*-1,6-(Hexanediyl)bis[*N′*-cyclohexyl-*N′*-[(cyclohexylimino)(4-morpholinyl)methyl]-*N*-methylurea] (71). Guanidine **3** (8.80 g, 30 mmol) and THF (60 mL) were cooled to −78 °C, and *n*-butyllithium (18.8 mL, 30 mmol, 1.6 M in hexane) was added. The reaction mixture was warmed to 0 °C, and after 1 h, bis(carbamoyl chloride) **75** (2.69 g, 10 mmol), dissolved in THF (10 mL), was added. After 16 h, the reaction was quenched with H_2O (100 mL). The aqueous portion was extracted with Et_2O (2 × 100 mL), and the organic portions were combined, dried, and concentrated in vacuo. The product was then chromatographed (SiO_2, eluted with Et_2O saturated with NH_3) to yield 2.30 g of pure material and less pure fractions. The less pure material was chromatographed on a Waters' Prep-500 (SiO_2, eluted with CH_2Cl_2 saturated with am-

Table XI. Physical and Analytical Data for Analogues

no.	formula	NMR ($CDCl_3$) δ	anal.	mp, °C	isolation[a,b,c]
21	$C_{42}H_{74}N_8O_2$	1.10–1.95 (m, 56 H), 3.01–3.46 (m, 14 H), 3.46–3.88 (br s, 2 H), 4.55 (t, 2 H)	CHN	108–113	product precipitated
22	$C_{44}H_{78}N_8O_2$	1.00–2.10 (m, 60 H), 3.11–3.40 (m, 14 H), 3.57–4.03 (m, 2 H), 4.65 (t, 2 H)	CHN	55–80	A
23	$C_{42}H_{74}N_8O_2S_2$	1.00–2.05 (m, 48 H), 2.46–2.69 (m, 8 H), 3.00–3.35 (m, 6 H), 3.40–3.95 (m, 10 H), 4.60 (t, 2 H)	CHN	100–120	product precipitated
24	$C_{58}H_{102}N_8O_2$	1.10–2.20 (m, 48 H), 2.80 (s, 12 H), 3.01–3.33 (m, 6 H), 3.52–3.96 (m, 2 H), 4.60 (t, 2 H)	CHN	115–119	product precipitated
25	$C_{58}H_{102}N_8O_2$	0.90–2.10 (m, 88 H), 2.75–3.70 (m, 12 H), 4.60 (t, 2 H)	CHN	73–110	A
26	$C_{58}H_{78}N_8O_2$	0.80–1.80 (m, 48 H), 3.10–3.60 (m, 8 H), 6.90–7.45 (m, 20 H), 9.40 (t, 2 H)	CHN	136–138	product precipitated
28	$C_{48}H_{62}N_8O_2$	1.10–1.65 (m, 20 H), 2.20 (s, 6 H), 2.35 (s, 6 H), 2.91–3.21 (m, 4 H), 3.31–3.61 (m, 8 H), 4.90 (br s, 2 H), 6.60–7.15 (m, 16 H)	CHN	97–102	product precipitated
29	$C_{46}H_{58}N_8O_2S_2$	1.15 (br s, 8 H), 2.20 (s, 6 H), 2.30 (s, 6 H), 2.42–2.68 (m, 8 H), 2.81–3.15 (m, 4 H), 3.62–3.88 (m, 8 H), 4.70 (br s, 2 H), 6.60–7.12 (m, 16 H)	CHN	130–131	product precipitated
30	$C_{46}H_{58}N_8O_4$	1.08–1.45 (m, 8 H), 2.20 (s, 3 H), 2.35 (s, 3 H), 2.95–3.26 (m, 4 H), 3.41–3.71 (m, 16 H), 4.80 (br s, 2 H), 6.55–7.20 (m, 16 H)	CHN	130–131	product precipitated
31	$C_{30}H_{58}N_8O_2$	1.00–2.00 (m, 40 H), 3.10–3.60 (m, 14 H), 3.81–4.21 (m, 2 H), 4.40 (t, 2 H)	CH	–	B
32	$C_{32}H_{62}N_8O_2$	0.90–1.80 (m, 44 H), 2.98–3.30 (m, 12 H), 3.30–3.70 (m, 2 H), 3.96–4.34 (m, 2 H), 4.60 (t, 2 H)	CHN	–	A
33	$C_{30}H_{58}N_8O_2S_2$	0.90–1.60 (m, 32 H), 2.48–2.70 (m, 8 H), 3.01–3.27 (m, 4 H), 3.44–3.68 (m, 10 H), 4.10 (s, 2 H), 4.60 (t, 2 H)	CHNS	–	C
34	$C_{30}H_{58}N_8O_4$	1.00–1.45 (m, 32 H), 3.05–3.36 (m, 12 H), 3.40–3.75 (m, 10 H), 3.90–4.38 (m, 2 H), 4.60 (br s, 2 H)	CHN	–	A
35	$C_{26}H_{54}N_8O_2$	0.90–1.80 (m, 32 H), 2.75 (m, 12 H), 3.00–3.60 (m, 6 H), 3.73–4.14 (m, 2 H), 4.50 (d, 2 H)	CHN	105–113	triturated with Et_2O
36	$C_{46}H_{86}N_8O_2$	0.90–1.90 (m, 72 H), 2.80–3.60 (m, 10 H), 3.78–4.04 (m, 2 H), 4.60 (t, 2 H)	CHN	–	A
40	$C_{40}H_{70}N_8O_4$	1.05–2.10 (m, 44 H), 3.08–3.55 (m, 14 H), 3.57–3.90 (m, 10 H), 4.58 (t, 2 H)	CHN	148–151	D
41	$C_{41}H_{72}N_8O_4$	1.00–2.10 (m, 46 H), 3.02–3.48 (m, 14 H), 3.61–3.86 (m, 10 H), 4.53 (t, 2 H)	CHN	67–95	A
42	$C_{43}H_{76}N_8O_4$	1.00–2.10 (m, 50 H), 3.02–3.38 (m, 14 H), 3.54–3.82 (m, 10 H), 4.50 (t, 2 H)	CHN	59–85	A
43	$C_{44}H_{78}N_8O_4$	1.05–2.10 (m, 52 H), 2.98–3.38 (m, 14 H), 3.52–3.86 (m, 10 H), 4.50 (t, 2 H)	CHN	133–135	F
44	$C_{45}H_{80}N_8O_4$	1.05–2.05 (m, 54 H), 3.00–3.41 (m, 14 H), 3.52–3.83 (m, 10 H), 4.52 (t, 2 H)	CHN	58–80	E
45	$C_{46}H_{82}N_8O_4$	1.00–2.10 (m, 56 H), 2.99–3.38 (m, 14 H), 3.51–3.85 (m, 10 H), 4.53 (t, 2 H)	CHN	136–139	F
46	$C_{21}H_{35}N_5O_3$	1.10–2.10 (m, 20 H), 3.05–3.90 (m, 14 H), 4.50 (br s, 1 H)	CHN	156–158	A, G
47	$C_{22}H_{37}N_5O_3$	0.90–2.10 (m, 22 H), 3.10–3.90 (m, 14 H), 6.05 (br s, 1 H)	CHN	160–164	A
48	$C_{22}H_{40}N_4O_2$	0.72–1.01 (m, 3 H), 1.01–2.10 (m, 24 H), 3.00–3.38 (m, 7 H), 3.52–3.88 (m, 5 H), 4.50 (t, 1 H)	CHN	100–105	F
55	$C_{42}H_{66}N_8O_4$	0.90–2.2 (m, 40 H), 3.10–3.80 (m, 20 H), 4.80 (br s, 2 H), 6.37–6.84 (m, 2 H), 6.98–7.36 (m, 2 H)	CHN	85–146	B
56	$C_{42}H_{66}N_8O_4$	0.90–2.10 (m, 40 H), 3.10–3.75 (m, 20 H), 4.70 (br s, 2 H), 6.70 (s, 4 H)	CHN	96–135	C
57	$C_{50}H_{74}N_8O_4$	1.00–2.10 (m, 40 H), 2.25 (s, 6 H), 3.20–4.85 (m, 20 H), 6.55 (br s, 2 H), 7.30–7.52 (m, 4 H) 8.00 (d, 2 H)	CHN	99–220	C
58	$C_{44}H_{70}N_8O_4$	0.90–2.10 (m, 40 H), 3.00–3.90 (m, 20 H), 4.35 (d, 4 H), 4.90 (t, 2 H), 7.11–7.42 (m, 4 H)	CHN	79–135	A
59	$C_{44}H_{70}N_8O_4$	0.90–2.10 (m, 40 H), 3.01–3.42 (m, 10 H), 3.57–4.10 (m, 10 H), 4.21–4.50 (m, 4 H), 4.70 (br s, 2 H), 7.19–7.54 (4 H)	CHN	83–121	C
60	$C_{63}H_{102}N_{12}O_6$	0.90–2.15 (m, 60 H), 2.96–3.33 (m, 15 H), 3.45–3.86 (m, 15 H), 4.17–5.00 (7, 9 H), 7.11–7.50 (m, 3 H)	CHN	100–134	E
61	$C_{42}H_{72}N_6O_4$	0.90–2.25 (m, 52 H), 2.95–3.40 (m, 10 H), 3.40–4.05 (m, 10 H)	CHN	116–120	F + H[c]
62	$C_{44}H_{76}N_6O_4$	1.00–2.20 (m, 56 H), 3.10–3.35 (m, 10 H), 3.60–3.90 (m, 10 H)	CHN	154–155	I[c]
63	$C_{36}H_{60}N_6O_4$	1.00–2.30 (m, 40 H), 3.00–3.95 (m, 20 H)	CHN	141–152	H[c]
64	$C_{42}H_{72}N_6O_6$	1.00–2.10 (m, 40 H), 2.95–4.30 (m, 24 H)	CHN	72–74	E[c]

[a] Chromatographed on SiO_2 and eluted with the following: A = E_2O/NH_3; B = Et_2O/MeOH (0.5%)/NH_3; C = Et_2O/hexane/NH_3; D = $Et_2O/CH_2Cl_2/NH_3$; E = CH_2Cl_2/NH_3. [b] Recrystallized from the following: F = CH_3CN; G = Et_2O; H = hexane; I = CH_2Cl_2. [c] These compounds were derived from the corresponding halide in Table V and not from an isocyanate.

Table XII. Physical and Analytical Data for Analogues

no.	formula	NMR ($CDCl_3$) δ	anal.	mp °C/ bp, °C (mm)	isolation[a]
66	$C_{21}H_{39}N_3O$	0.80–2.10 (m, 27 H), 2.75–3.50 (m, 8 H), 3.60–3.85 (m, 4 H)	high-res MS	145 (0.6)	distilled[b]
67	$C_{18}H_{33}N_3O$	1.00–1.90 (m, 20 H), 2.55 (s, 0.66 of 3 H, CH_3), 2.65 (s, 0.33 of 3 H, CH_3), 3.00–3.50 (m, 6 H), 4.52–4.85 (m, 4 H)	CHN	125 (0.4)	distilled[b]
70	$C_{40}H_{68}N_8O_4$	0.90–2.30 (m, 40 H), 2.80–3.75 (m, 28 H)	CHN	105–107	A[c]
72	$C_{40}H_{70}N_8O_4$	0.94–2.40 (m, 40 H), 2.80 (s, 6 H), 2.97–3.92 (m, 28 H)	CHN	85–115	B[c]
73	$C_{46}H_{74}N_8O_4$	0.97–2.30 (m, 40 H), 2.62 (s, 6 H), 2.95–3.40 (m, 12 H), 3.44–3.75 (m, 8 H), 4.35 (s, 4 H), 7.09–7.35 (m, 4 H)	CHN	102–135	C[c]

[a] Chromatographed on SiO_2 and eluted with the following: A = CH_2Cl_2/NH_3; B = CH_2Cl_2/hezne/NH_3; C = Et_2O/MeOH (2%)/NH_3. [b] These compounds were prepared by method C. [c] These compounds were prepared by method D.

monia) to afford an additional 2.90 g of product. The material was combined to yield 5.20 g (66%) of **71**: mp 63–114 °C; ^1H NMR ($CDCl_3$) δ 1.00–2.43 (m, 48 H), 2.73 (s, 6 H), 2.95–3.47 (m, 16 H), 3.47–3.80 (m, 8 H). Anal. ($C_{44}H_{78}N_8O_4$) C, H, N.

In Vitro Assay. Human blood was obtained from the antecubital vein and treated with sodium citrate (1 part of 3.8% sodium citrate to 9 parts of blood). Platelet-rich plasma (PRP) was prepared by centrifugation of the citrated blood at 100*g* for 10 min at 10 °C. Platelet count was adjusted with autologous platelet-poor plasma (PPP) to 3×10^5 platelets/mm^3.

Rats and guinea pigs were anesthetized with 1–2 cm^3 of 2.5% sodium cyclopal, i.p. Once the animals were unconscious, the abdomen was opened and blood was drawn from the abdominal aorta into a syringe containing citrate solution. Final citrate concentration was 0.38% for guinea pigs and 0.22% for rats. The blood was placed into tubes and centrifuged for 10 min at 100*g* at 10 °C. The PRP was pipetted off, and the remainder of the blood was centrifuged (1500*g* for 10 min at 10 °C) to obtain PPP.

Table XIII. Percent Inhibition of ADP-Induced Platelet Aggregation Data for Compounds 1 and 58

		% inhibn		
expt	concn of compd[a]	PGE_1	1	58
1	32	94		
	10	56	82	
	3.2	26	38	
	1.0		29	
2	32	90		
	10	28	85	
	3.2	1	62	
	1.0		27	
3	32	72		
	10	37		68
	3.2	14		40
	1.0			27

[a] The concentration of PGE_1 is in ng/mL; the concentration of 1 and 58 is in μg/mL.

The platelet count for the guinea pig plasma was adjusted to 3.5×10^5 platelets/mm^3 and for the rat plasma to 5.0×10^5 platelets/mm^3 with autologous PPP.

An ADP concentration required to give slightly less than maximal aggregation was used to test compounds for inhibition of aggregation with the use of a Payton aggregation module coupled with an Omniscribe recorder. The concentrations of compounds in Table VIII represent those that gave approximately 50% inhibition (IC_{50}). The approximate IC_{50} of these compounds on ADP-induced platelet aggregation was obtained at $^1/_2$ log intervals of drug concentration. PGE_1 was used as an internal standard to adjust for individual variation in platelet sensitivity. Table XIII illustrates typical studies on different days for the first two compounds in Table VIII. No correction for platelet sensitivity was necessary as the IC_{50} of the standard PGE_1 was consistently between 10 and 32 ng/mL. Compounds that did not display approximately 50% inhibition at 10 μg/mL were considered inactive.

To test for inactivation of the drug by the blood, compounds active in the in vitro screen were incubated in whole blood (10 μg/mL) at room temperature for 0, 1, and 2 h. PRP was prepared from an aliquot of the blood and was tested for inhibition of platelet aggregation. Control aggregation studies were performed with blood incubated with solvent used to dissolve the drug.

Ex Vivo Studies. Compounds were dissolved in Emulphor/ethanol/Tyrodes and were administered gastrically to rats and guinea pigs via gavage. At 2 h after drug or placebo administration, blood samples were drawn from all animals as described previously. PRP was prepared and aggregation experiments were conducted as previously described. Activity was determined by demonstration of a p value of <0.05 (Student's t test) for inhibition of ADP-induced platelet aggregation in PRP with respect to placebo.

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Synthesis of Novel Bifunctional Chelators and Their Use in Preparing Monoclonal Antibody Conjugates for Tumor Targeting

David A. Westerberg, Patrick L. Carney, Patrick E. Rogers, Steven J. Kline, and David K. Johnson*

Abbott Laboratories, Department 90M, Abbott Park, Illinois 60064. *Received March 24, 1988*

Bifunctional derivatives of the chelating agents ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid, in which a *p*-isothiocyanatobenzyl moiety is attached at the methylene carbon atom of one carboxymethyl arm, was synthesized by reductive alkylation of the relevant polyamine with (*p*-nitrophenyl)pyruvic acid followed by carboxymethylation, reduction of the nitro group, and reaction with thiophosgene. The resulting isothiocyanate derivatives reacted with monoclonal antibody B72.3 to give antibody–chelator conjugates containing 3 mol of chelator per mole of immunoglobulin, without significant loss of immunological activity. Such conjugates, labeled with the radioisotopic metal indium-111, selectively bound a human colorectal carcinoma implanted in nude mice when given intravenously. Uptake into normal tissues was comparable to or lower than that reported for analogous conjugates with known bifunctional chelators. It is concluded that substitution with a protein reactive group at this position in polyaminopolycarboxylate chelators does not alter the chelating properties of these molecules to a sufficient extent to adversely affect biodistribution and thus provides a general method for the synthesis of such chelators.

Current efforts to use monoclonal antibodies as a delivery system for targeting drugs, toxins, or radioisotopes to tumor foci in vivo[1-4] have spurred interest in improved methods for preparing such immunoconjugates. The potential utility of radioisotope conjugates in both the detection and treatment of neoplastic disease has, in particular, led to increasing work on methods for linking ra-

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