The 2-Chloro-3-indenylmethyloxycarbonyl and Benz[f]inden-3-ylmethyloxycarbonyl Base-Sensitive Amino-Protecting Groups. Application to an Inverse Merrifield Approach to Peptide Synthesis[1]

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Two new base-labile amino-protecting groups, which are more sensitive than the FMOC function, are described: the 2-chloro-3-indenylmethyloxycarbonyl (CLIMOC) and benz[f]inden-3-ylmethyloxycarbonyl (BIMOC) groups. The former was determined to be usable in solvents such as methylene dichloride but not in DMF, the latter in any common solvent including DMF. Key intermediate alcohols **10** and **16** were synthesized from 2-chloroindene (**9**) and benz[f]indene (**14**). Treatment of indene with chlorine gave **8**, which upon dehydrochlorination with DMF gave **9**, which was then converted to **10** by a standard procedure involving formylation and reduction. Benz[f]indene was converted to its anion by means of *n*-butyllithium and the anion hydroxymethylated by gaseous formaldehyde. The alcohols were converted to the corresponding chloroformates and thence to succinimido ester **12** and azidoformate **18** for clean, selective protection of amino acids. Model CLIMOC- and BIMOC-amino acids were synthesized and demonstrated to be useful in carrying out a continuous peptide synthesis via a two-polymer (polymeric reagents) approach. The protected amino acids were first loaded onto a phenolic polymer such as **21**, and the resulting polymeric active esters were used to acylate an amino acid ester or peptide ester. The resulting protected peptide esters were deblocked via silica-based reagents **6** or **23**. The acylation step was then repeated with the next amino acid, and the synthesis continued in the same way until completed. Tetrapeptide **26** and pentapeptide **27** were synthesized in this way via CLIMOC (CH_2Cl_2) and BIMOC (DMF) protection, respectively. These represent the first examples of clean, continuous two-polymer syntheses carried out in a single solvent without the release of any low molecular weight byproducts into the solution.

In spite of the high level to which the normal Merrifield method[2] of solid-phase peptide synthesis has been brought, there still is reason to probe an alternate technique in which two polymers or supports are applied sequentially, one to effect acylation and the other to effect deblocking, with the growing peptide remaining always in solution.[3,4] Possible advantages for such an approach vs the normal technique result from the fact that facile monitoring for completion of both acylation and deblocking steps as well as continuous assessment of product purity is possible by spectroscopic or chromatographic methods. If justified by the analytical data, the process could be discontinued at any time, the peptide purified, and the synthesis continued. In the normal technique, any impurities or error peptides built up on the resin support can only be removed at the end of the synthesis.

Disadvantages of the inverse method include problems that may arise due to possible insolubility[5] as the chain is lengthened, irreversible absorption of even soluble peptides onto the polymeric reagent, dilution of the reaction medium due to extensive washing, etc. In order to begin an investigation of some of these potential problems and to see how far one can go in the chain-lengthening process by this technique, we initiated a study of the "two-support" or "polymeric reagents" approach to continuous peptide synthesis. If warranted, fully automated syntheses seem feasible, with reactions proceeding batchwise or in columns or cartridges.

Although prior to this work a large number of useful polymeric acylating agents had been developed,[3] no suitable deblocking polymers had been devised that did not require major changes in the reaction medium upon proceeding from the acylation to the deblocking step. For eventual full automation, the common acid-sensitive amino-protecting groups are not convenient since deblocking must be followed by treatment with a base to liberate the free amino peptide.[6] The base must then be removed prior to the subsequent deblocking step. The closest approach to date to a continuous system involved the use of sulfenyl α-amino protection, but solvent changes were required at each stage, and the deblocking step was marred

(1) A number of abbreviations are used in this paper. Those for natural amino acids and peptides follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* 1971, *247*, 997). Other abbreviations are as follows: TFA = trifluoroacetic acid, Fl = fluorene, FM = 9-fluorenylmethyl, FMOC = (9-fluorenylmethoxy)carbonyl, DCC = dicyclohexylcarbodiimide, HOBt = *N*-hydroxybenzotriazole, HOSu = *N*-hydroxysuccinimide, PCA = *p*-chloroaniline, Phg = α-phenylglycine, DBF = dibenzofulvene, IMOC = 3-indenylmethyloxycarbonyl, CLIMOC = 2-chloro-3-indenylmethyloxycarbonyl, BIMOC = Bz[f]-3-IMOC = benz[f]inden-3-ylmethyloxycarbonyl.

(2) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1980; Vol. 2, Part A, p 1.

(3) For reviews, see: (a) Fridkin, M. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1980; Vol. 2, Part A, p 333. (b) Patchornik, A.; Cohen, B. J. In *Perspectives in Peptide Chemistry*; Eberle, A., Geiger, R., Wieland, T., Eds.; Karger: Basel, 1981; p 118.

(4) An alternative two-polymer technique, basically a hybrid of the normal and inverse Merrifield methods (Shadchan approach), has been described recently. See: (a) Shai, Y.; Jacobson, K. A.; Patchornik, A. *J. Am. Chem. Soc.* 1985, *107*, 4249. (b) Patchornik, A. *CHEMTECH* 1987, 58.

(5) For new developments in solubility enhancement during peptide synthesis and new insights into the general problem of peptide solubility and/or aggregation effects, see: (a) Voelter, W.; Müller, J. *Liebigs Ann. Chem.* 1983, 248. (b) Eckert, H.; Seidel, C. *Angew. Chem.* 1986, *98*, 168. (c) Rizo, J.; Albericio, F.; Romero, G.; Garcia-Echeverria, C.; Claret, J.; Muller, C.; Giralt, E.; Pedroso, E. *J. Org. Chem.* 1988, *53*, 5386. (d) Narita, M.; Honda, S.; Umeyama, H.; Obana, S. *Bull. Chem. Soc. Jpn.* 1988, *61*, 281. (e) Narita, M.; Doi, M.; Nakai, T.; Takegahara, H. *Int. J. Pept. Protein Res.* 1988, *32*, 200. (f) Narita, M.; Ogura, T.; Sato, K.; Honda, S. *Bull. Chem. Soc. Jpn.* 1986, *59*, 2433. (g) Toniolo, C.; Bonora, G. M.; Heimer, E. P.; Felix, A. M. *Int. J. Pept. Protein Res.* 1987, *30*, 232. (h) Maser, F.; Altmann, K.-H.; Mutter, M.; Toniolo, C.; Bonora, G. M. *Biopolymers* 1985, *24*, 1057. (i) Ueki, M.; Saito, T.; Sasaya, J.; Ikeda, S.; Oyamada, H. *Bull. Chem. Soc. Jpn.* 1988, *61*, 3653.

(6) Compare: Stern, M.; Kalir, R.; Patchornik, A.; Warshawsky, A.; Fridkin, M. *J. Solid-Phase Biochem.* 1977, *2*, 131.

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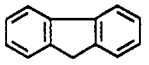
Table I. Acidity of Protecting Group Parents (pK_a)



22.6

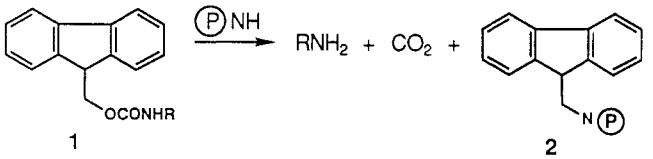
2-Me	23.1
9-Me	22.3
2-Cl	20.2
2,7-Br_2	17.9

20.1

2-Me	21.8
3-Me	22.5

by the release of low molecular weight side products into the solution.[7]

With recent developments in base-sensitive amino-protecting groups such as the FMOC function[8] which upon deblocking lead directly to the formation of a *free* amino peptide, it appeared that an ideal solution to the deblocking problem might be at hand. Indeed, attempts to develop polymeric reagents for FMOC deblocking were made in two laboratories, but deblocking times proved to be longer than desirable, and more importantly, the FMOC-deblocking byproduct, DBF, could not be completely scavenged by the deblocking amine (eq 1).[9,10] If



1 2

P = polystyrene, polyamide, or silica support eq. 1

not completely removed, DBF may polymerize during the synthesis, setting to a gel, which could clog a column or impede elution of the desired peptide. Reaction of DBF with a cyclic secondary amine is at least 1 order of magnitude slower than deblocking of the FMOC function by the same amine.

A major goal of the present work was to uncover new types of base-sensitive amino-protecting groups which could be more efficiently deblocked than the FMOC function and for which the resulting byproducts would be scavenged completely or nearly completely by suitable polymeric reagents. The first substrates examined, which are described in the present paper, were direct analogues of the FMOC system. A measure of both deblocking and scavenging rates should be the relative pK_a's of the parent hydrocarbons from which the protecting groups are derived (Table I).[11] First examined were derivatives of 2,7-dichlorofluorene, but unfortunately, while deblocking reactions were significantly faster, this was not true for scavenging reactions, possibly because of constant steric factors.

A protecting group based on indene seemed a plausible candidate for our purposes in view of the expected acidity

Scheme I

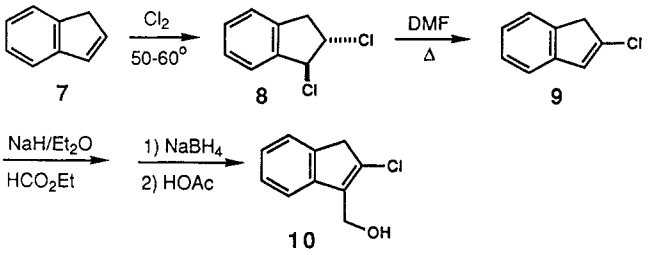
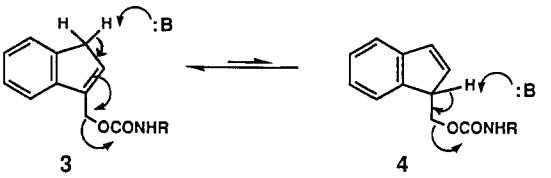


Table II. Treatment of Base-Sensitive Urethanes (X-PCA) with Piperidine[a]

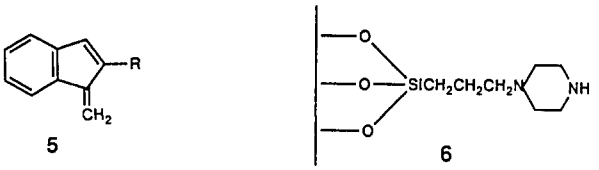
X	deblocking[b]	scavenging[b]
IMOC	<90	<90
CLIMOC	<10	<10
BIMOC	<14 h	<14 h
FMOC	18 h	>2 weeks

[a] The urethane (0.1 mmol) in 5 mL of $CHCl_3$ was treated with 0.2 mL of piperidine and the solution allowed to stand at room temperature. Reactions were monitored for completeness by TLC. [b] Time in minutes unless specified otherwise.

(Table I) and the openness for attack, especially in the case of the desired achiral thermodynamically preferred 3-isomer of the appropriate urethane **3**, even by a hindered base. In fact, model IMOC derivatives, e.g., IMOC-PCA



(**3**, R = C_6H_4Cl-*p*), were deblocked about 10 times faster than FMOC analogues, and more importantly, the scavenging reactions were about 250 times as fast. Scavenging is so fast that for all practical purposes it is complete at the time of deblocking and only traces of the benzofulvene byproduct **5** (R = H) remain in solution on treatment of an IMOC derivative with a polymeric amine such as the piperazino-loaded silica derivative **6**. Unfortunately, except for the glycine derivative, most IMOC-amino acid derivatives synthesized to date proved to be oils that were difficult or impossible to crystallize. More satisfactory results were obtained with amino acids derived from the 2-chloro and 5,6-benzo analogues of indene. The 2-chloro-3-indenylmethyloxycarbonyl (CLIMOC) system is readily available from indene according to reactions outlined in Scheme I for preparation of key alcohol **10**. The inductive effect of the 2-chloro substituent (pK_a data not available) makes urethane derivatives of **10** even more reactive than the IMOC analogues. Data comparing deblocking and scavenging reactions are presented in Table II. Upon addition of a deblocking amine to a solution of CLIMOC-PCA, a yellow color develops which fades as scavenging occurs. This visual indication of the course of the reaction is presumably due to the color of **5** (R = Cl).



Crystalline CLIMOC-protected amino acids could be synthesized in the usual manner by using chloroformate **11** or, better, *N*-hydroxysuccinimide ester **12**, the latter for

(7) (a) Stern, M.; Fridkin, M.; Warshawsky, A. *J. Polym. Sci., Polym. Chem. Ed.* **1982**, *20*, 1469. (b) Stern, M.; Warshawsky, A.; Fridkin, M. *Int. J. Pept. Protein Res.* **1981**, *17*, 531. (c) Stern, M.; Warshawsky, A.; Fridkin, M. *Int. J. Pept. Protein Res.* **1979**, *13*, 315.

(8) (a) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401. (b) Atherton, E.; Sheppard, R. C. In *The Peptides*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: New York, 1987; Vol. 9, Part C, p 1.

(9) (a) Carpino, L. A.; Mansour, E. M. E.; Cheng, C. H.; Williams, J. R.; MacDonald, R.; Knapczyk, J.; Carman, M.; Lopusinski, A. *J. Org. Chem.* **1983**, *48*, 661. (b) Carpino, L. A.; Mansour, E. M. E.; Knapczyk, J. *J. Org. Chem.* **1983**, *48*, 666.

(10) Arshady, R.; Atherton, E.; Sheppard, R. C. *Tetrahedron Lett.* **1979**, 1521.

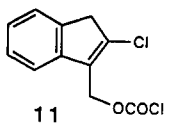
(11) (a) Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456. (b) Bordwell, F. G.; McCollum, G. J. *J. Org. Chem.* **1976**, *41*, 2391.

Table III. Characterization of Protected Carbanilates

compd[a]	yield, %	mp, °C (recrystn solv)	^{1}H NMR:[b] δ	mol formula	anal. data: calcd/found C	H	N
IMOC-PCA[c]	75	116 (Et_2O)	3.35 (m, 2, CH_2), 5.20 (s, 2, CH_2O), 6.50 (br, 1, CH=), 6.85 (s, 1, NH), 7.25–7.45 (m, 8, aryl)[d]	$C_{17}H_{14}ClNO_2$	68.11/68.25	4.67/4.84	4.67/4.44
CLIMOC-PCA	93	152 ($CHCl_3$)	3.55 (s, 2, CH_2), 5.55 (t, 2, CH_2O), 6.75 (s, 1, NH), 7.15–7.50 (m, 8, aryl)	$C_{17}H_{13}Cl_2NO_2$	61.09/61.12	3.92/3.80	4.19/3.95
BIMOC-PCA	87	177–8 (benzene–hexane)	3.45 (m, 2, CH_2Ar), 5.25 (m, 2, CH_2O), 6.60 (m, 1, CH=), 6.75 (br, 1, NH), 7.15–8.05 (m, 10, aryl)	$C_{21}H_{16}ClNO_2$	72.10/72.30	4.61/4.74	4.00/3.87
Bz[*f*]IMOC-1-PCA[e]	87	157–8 (benzene–hexane)	3.60–4.70 (m, 3, $CHCH_2$), 6.40–6.60 (m, 1, 2-CH=), 6.55 (br, 1, NH), 6.85–7.05 (m, 1, 3-CH=), 7.15–8.05 (m, 10, aryl)	$C_{21}H_{16}ClNO_2$	72.10/72.08	4.61/4.66	4.00/3.93

[a] Prepared by refluxing 0.02 mol of the alcohol and 0.02 mol of *p*-chlorophenyl isocyanate in 15 mL of benzene for 4–8 h followed by recrystallization from an appropriate solvent. [b] Solvent $CDCl_3$. [c] The precursor indene-3-methanol, mp 62–3 °C, was prepared by the method described for benz[*f*]indene-3-methanol, the initial crude mixture of 1- and 3-isomers being isomerized to the 3-isomer by Et_3N–EtOH. See also: Friedrich, E. C.; Taggart, D. B. *J. Org. Chem.* **1975**, *40*, 720. [d] NH peak at δ 9.8 in DMSO-d_6. [e] Derived from benz[*f*]-indene-1-methanol (**15**).

avoidance of dipeptides or higher peptides when free amino acids are acylated.[12-14] As will be noted later in this



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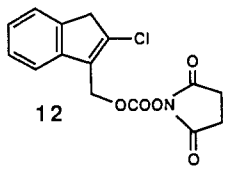

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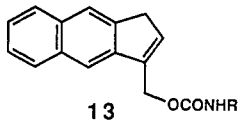
discussion, these CLIMOC derivatives have been successfully used to effect two-support syntheses of simple model peptides. CLIMOC intermediates proved readily soluble in methylene dichloride, which was used exclusively as a convenient and inexpensive reaction medium. However, because methylene dichloride is relatively nonpolar, it is not certain whether higher protected peptides will continue to show the requisite solubility in this solvent. Attempts to use CLIMOC derivatives in the more generally useful solvent DMF foundered when it was noted that these derivatives were not completely stable in DMF and other dipolar aprotic solvents. It therefore appeared useful to search for a protective system with a sensitivity profile somewhat intermediate between that of the FMOC and CLIMOC functions.

Out of this search came the BIMOC system **13**, which is derived from the recently described fluorene isomer, benz[*f*]indene (**14**).[15] Although benz[*f*]indene could be



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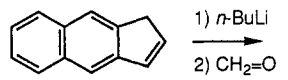
converted to the desired alcohol **16** by our standard technique of formylation followed by borohydride reduction,[16] yields were not as high as achieved by an alternate technique (Scheme II) involving direct hydroxymethylation



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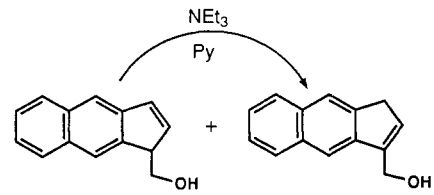
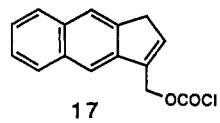


15 16

through the *n*-butyllithium-generated anion. This method gave a 95:5 mixture of **15** and **16** with the undesired isomer **15**, bearing an asymmetric center, predominating. Base-catalyzed isomerization, however, leads to the desired alcohol **16**, from which the corresponding chloroformate **17** and azidoformate **18** were readily prepared. The latter



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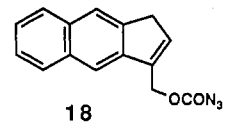

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was used to acylate amino acids without oligomer formation.[12-14] Initial studies compared the BIMOC-PCA derivatives obtained from **16** with analogous FMOC, IMOC, and CLIMOC systems (Tables II and III). In contrast to CLIMOC systems, the BIMOC analogues were stable in DMF solution for at least 24 h. Similar stability is also shown in pyridine. Both CLIMOC and BIMOC systems are relatively stable to acidic reagents, although not to the same degree as FMOC analogues. With these two new base-sensitive protecting groups available, we set out to demonstrate their applicability to two-support syntheses in methylene dichloride and DMF, respectively. For this purpose, efficient acylating and deblocking polymers or supports were needed.

Acylating Polymers

Among the many appropriate polymers that have been described in the literature, we selected for initial study active esters derived from 4-hydroxy-3-nitrobenzophenone.[17] In addition, a new, more effective phenolic

(12) Compare: Tessier, M.; Albericio, F.; Pedroso, E.; Grandas, A.; Eritja, R.; Giralt, E.; Granier, C.; van Rietschoten, J. *Int. J. Pept. Protein Res.* **1983**, *22*, 125.
(13) Lapatsanis, L.; Milias, G.; Froussios, K.; Kolovos, M. *Synthesis* **1983**, 671.
(14) Sigler, G. F.; Fuller, W. D.; Chaturvedi, N. C.; Goodman, M.; Verlander, M. *Biopolymers* **1983**, *22*, 2157.
(15) Carpino, L. A.; Lin, Y.-Z. *J. Org. Chem.*, preceding paper in this issue.
(16) Carpino, L. A. *J. Org. Chem.* **1980**, *45*, 4250.

Scheme III

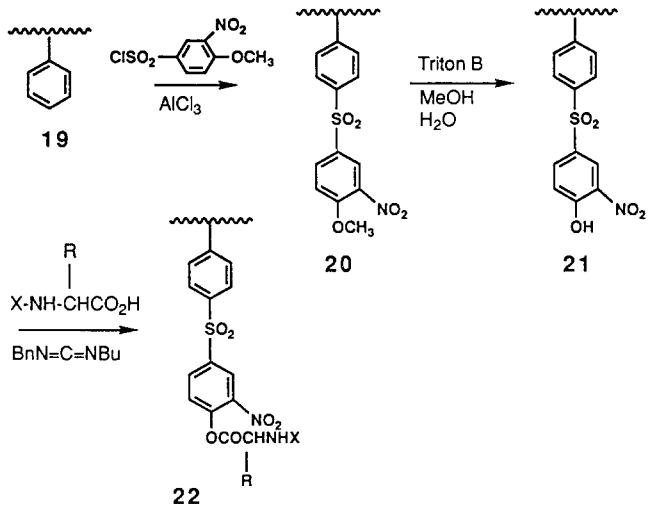
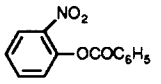
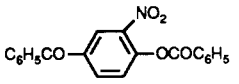
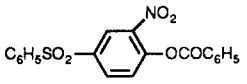


Table IV. Relative Rates for Acylation of t-BuNH$_2$[a]

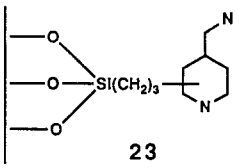
	k_{rel}
	1
	40
	230

[a] Each active ester (1 mmol) was dissolved in 50 mL of $CHCl_3$ and 4 mL of t-BuNH$_2$ added. Completion of the reaction was monitored by TLC.

polymer, **21**, was obtained via the outline presented in Scheme III. Phenol **21** and the analogous carbonyl derivative were loaded with both CLIMOC- and BIMOC-amino acids by using N-benzyl-N'-butylcarbodiimide as a convenient coupling agent whose derived urea is easily washed away from the polymeric reagent.[18] Loadings of 0.5–0.7 mmol/g were achieved. The reactivities of low molecular weight analogues of these two polymeric active esters are compared in Table IV.

Deblocking Polymers

For the CLIMOC system, reagent **6**, bearing piperazino units on the surface of silica gel,[9b] proved to be an effective deblocking agent. Because of its lesser reactivity, the BIMOC system demanded a more reactive agent. This was found in a comparable amine **23** derived from 4-(amino-



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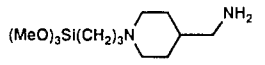
methyl)piperidine, which was prepared in a manner similar to that previously reported for **6**. In this case, attachment of the amine to the silica gel surface can take place through

Table V. Deblocking of BIMOC-PCA[a]

amount of substrate, mg	solvent (amount)	result
270	pyridine (3 mL)	no reaction, 24 h
70	piperidine (0.7 mL)	complete deblocking and scavenging after 5 min
34.9	piperidine (0.2 mL) plus $CHCl_3$ (5 mL)	complete deblocking and scavenging after 14 h
11.7	DMF (1.5 mL) plus 0.7 g of piperazyl silica **6**	complete deblocking in 3 h, scavenging in 3.5 h
11.7	CH_2Cl_2 (1.5 mL) plus 0.7 g of piperazyl silica **6**	deblocking incomplete after 24 h
11.7	DMF (1.5 mL) plus 0.7 g of piperidino silica **23**	deblocking and scavenging complete after 30 min[b]
11.7	CH_2Cl_2 (1.5 mL) plus 0.7 g of piperidino silica **23**	deblocking and scavenging complete after 10 h[b]

[a] Solutions of the materials and amounts indicated were allowed to stand at room temperature or, in the case of polymer reagents, rotated at room temperature. Reactions were monitored by TLC. [b] A trace of the fulvene remained.

both the primary and secondary sites, the intermediate silane being probably a mixture of **24** and **25**. As shown



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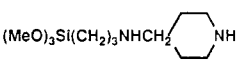

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by model comparison studies with 4-(aminomethyl)-piperidine and (aminomethyl)cyclohexane, the primary amino function acts as a scavenging site as well as the cyclic secondary amino group. Comparisons of the reactivity in various solvents of silica-based deblocking agents **6** and **23** are collected in Table V. The marked increase in reactivity in DMF relative to methylene dichloride is striking.

Two-Support Peptide Synthesis

With all necessary elements available, we were able to demonstrate a continuous two-support peptide synthesis for the first time. Via a manual batch technique without purification of any intermediates, the tetrapeptide **26** was

CLIMOC-Gly-Gly-Phe-Leu-OBn
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assembled in an overall yield of 74% from leucine benzyl ester in methylene dichloride solution, using CLIMOC protection throughout with deblocking via piperazino reagent **6**. A model racemization study involving loading of CLIMOC-phenylalanine onto the phenolic resin, coupling with leucine methyl ester, deblocking via **6**, and finally N-benzoylation to give the corresponding benzoyl dipeptide ester showed by HPLC analysis that less than 0.3% racemization had occurred at the phenylalanine residue.[19]

With BIMOC protection, an analogous synthesis of pentapeptide **27** was carried out in DMF solution. Acy-

FMOC-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn
27

lation steps were allowed to proceed for 1 h, although TLC analysis indicated that the reactions were complete after about 30 min. Each deblocking step, using silica reagent **23**, required about 3.5 h for completion. In each step the polymer was filtered and washed with methylene dichloride so that upon evaporation of the volatile solvent the original volume of the more effective medium, DMF, would be restored. Thorough removal of all methylene

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dichloride is important since, as already noted in connection with Table V, deblocking reactions are more sluggish if any significant amount of this solvent remains in the final DMF solution. The last acylation step was carried out with the appropriate FMOC derivative since the product, peptide **27**, had been prepared previously by another method and had been fully characterized.[20] Although this initial synthesis, which gave a yield of only 34%, was not optimized, the basic approach has been validated. Significant losses may have occurred during the washing steps.

Conclusions

It has been shown that continuous manual assembly of short peptides is possible by the tandem use of two polymeric reagents. A next step will be to examine a mechanized batch synthesis or reactions in columns via continuous flow techniques, the latter option providing for greater efficiency and easier on-line monitoring. Further rationalization to avoid the differential swelling of polystyrene reagents suggests use of a silica-based matrix for the acylation as well as the deblocking step. Although in this study the polymeric reagents were not demonstrated to be reusable, this is expected to be the case. The acylating agents may simply be recharged with the same amino acid, the deblocking agents reactivated via treatment with piperidine as has already been demonstrated for FM-substituted analogues. Indeed both reagents should be fully regenerated by treatment with excess piperidine. Finally it should be pointed out that the two new base-sensitive amino-protecting groups described here also show promise for use in ordinary Merrifield syntheses, the CLIMOC derivatives in methylene dichloride and the BIMOC analogues in any solvent, including DMF, as replacements for the FMOC system in specific instances where the increased speed of deblocking could be advantageous. Possible examples might be chain elongation steps during which base-catalyzed side reactions such as diketopiperazine,[21] aminosuccinimide,[22] or pyroglutamine[23] formation might be occurring.

Experimental Section

Instrumentation and General Procedures. Melting points and boiling points are uncorrected, the former being obtained with a Mel-Temp apparatus. Infrared spectra were determined on Perkin-Elmer Model 237B or 1310 spectrometers and ^{1}H NMR spectra on Perkin-Elmer R-12 (60 MHz) or R-32 (90 MHz) or Varian XL-200 (200 MHz) or XL-300 (300 MHz) instruments with Me_4Si as internal standard. All ^{13}C NMR spectra were recorded on a Varian Model XL-300 spectrometer at 75 MHz. Elemental analyses were carried out by the University of Massachusetts Microanalytical Laboratory under the direction of Greg Dabkowski. HPLC data were obtained with a Waters automated system incorporating a Model 721 system controller, 730 data module, U6K injector, 45 and 6000 solvent delivery systems, a Model 441 UV detector, and a Z-module radial compression unit. Optical rotations were obtained on a Rudolph Autopol III digital polarimeter. In order to avoid disintegration of the beads, all reactions involving polymeric reagents (polystyrene or silica) were carried out in simple, round-bottomed flasks fitted to the shaft of a rotary evaporator, the vessel being rotated for the appropriate time.

2-Chloroindene. Two hundred grams of indene (bp 181–2 °C) was added to a 1-L, three-necked, round-bottomed flask fitted with a thermometer. Chlorine gas was passed through the indene by using a gas dispersion tube at a rate such that the temperature remained at about 60 ± 5 °C. After about 1 h or longer, depending on the rate of chlorine addition, the temperature dropped to 30–5 °C, indicating completion of the reaction, which is also signaled by disappearance of the complex multiplets at δ 6.3 and 6.6 in the ^{1}H NMR spectrum and their replacement by a clean triplet at δ 6.6. The mixture was transferred to a 2-L flask, 600 mL of DMF was added, and the solution was refluxed for 2 h. The dark reaction mixture was poured onto 600 g of ice, the organic layer removed, and the aqueous layer extracted with two 75-mL portions of Skelly F. The combined extracts were washed with two 200-mL portions of water and dried ($MgSO_4$), and the solvent was removed in vacuo. Distillation gave 204 g (77.9%) of the chloroindene: bp 100–10 °C (10 mm) or 37 °C (0.1 mm) [lit.[24] bp 95–8 °C (11 mm)]; ^{1}H NMR ($CDCl_3$) δ 3.3 (d, 2, CH_2), 6.65 (t, 1, CH=), 7.2 (m, 4, aryl). Storage of pure 2-chloroindene in the freezer as a solid prevents decomposition with development of a yellow color.

2-Chloroindene-3-methanol (10). Some investigators have experienced difficulty in duplicating the reported yield for this preparation. The originator of the method (B.J.C.) suggests that for best results the preparation should be completed as quickly as possible up to the point of obtaining the crude alcohol. A flask was charged with 75 g of 2-chloroindene, 50 mL of ethyl formate, 400 mL of dry ether, and 23 g of NaH (55–60% in oil). The mixture was heated to reflux to initiate the reaction as indicated by a vigorous evolution of hydrogen. At this point the flask was cooled in an ice–water bath until H_2 evolution subsided and then refluxed again for an additional 10 min. Excess NaH was decomposed with water, and more water was added to give two layers. The aldehyde that had formed dissolved in the basic aqueous solution, which was extracted several times with ether. To the aqueous layer in a round-bottomed flask was added 19 g of $NaBH_4$. With vigorous stirring and cooling in an ice–water bath, glacial HOAc was added dropwise at the rate of about one drop per second until the mixture became acidic. The alcohol was extracted with ether and the organic solution dried ($MgSO_4$) and evaporated. The crude alcohol (75 g, 83%) was recrystallized from CCl_4 to give white crystals: mp 104–5 °C; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 1, OH), 3.50 (s, 2, CH_2), 4.65 (t, 2, CH_2O), 7.1–7.6 (m, 4, aryl).

Anal. Calcd for $C_{10}H_9ClO$: C, 66.49; H, 5.02; Cl, 19.63. Found: C, 66.20; H, 5.02; Cl, 19.90.

In case the crude alcohol was obtained as an oily solid, the solid portion (about half of the material) was collected separately and recrystallized from CCl_4 while the oil was chromatographed on silica gel by using CH_2Cl_2–hexane (2:1) as eluent.

(2-Chloroinden-3-yl)methyl Chloroformate (11). A solution of 18 g of alcohol **10** in 100 mL of dry THF was cooled to 0 °C, 30 g of phosgene added, and the mixture stirred at 0 °C for 3 h. Excess phosgene and solvent were removed under water pump vacuum at 0 °C, and the residue was recrystallized from hexane to give 22 g (91%) of the chloroformate: mp 52–3 °C; ^{1}H NMR ($CDCl_3$) δ 3.5 (s, 2, CH_2), 5.25 (s, 2, CH_2O), 7.15–7.40 (m, 4, aryl).

Anal. Calcd for $C_{11}H_8Cl_2O_2$: C, 54.35; H, 3.32; Cl, 29.17. Found: C, 54.63; H, 3.17; Cl, 28.95.

(2-Chloroinden-3-yl)methyl Succinimido Carbonate (12). To a solution of 18.4 g of **11** in 200 mL of $CHCl_3$ was added portionwise with stirring over a half-hour period 22.4 g of the dicyclohexylamine salt of *N*-hydroxysuccinimide.[25] The mixture was stirred overnight at room temperature and filtered and the filtrate washed twice each with 10% citric acid, 10% $NaHCO_3$, and water. Removal of solvent from the dried ($MgSO_4$) solution gave a gold-colored oily residue, which was recrystallized from $CHCl_3$–Et_2O to give 24.1 g (80%) of the carbonate: mp 152–4

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°C; ^{1}H NMR ($CDCl_3$) δ 2.8 (s, 4, CH_2CH_2), 3.6 (br s, 2, CH_2), 5.4 (br s, 2, CH_2O), 7.4 (br s, 4, aryl).

Anal. Calcd for $C_{15}H_{12}ClNO_5$: C, 55.90; H, 3.73; Cl, 11.02. Found: C, 55.91; H, 3.70; Cl, 10.97.

***N*-(2-Chloroinden-3-ylmethyloxycarbonyl)phenylalanine.** Phenylalanine (8.25 g) was dissolved in 150 mL of 0.6 N Na_2CO_3 and the solution cooled to 10 °C. A cold solution of 13 g of **11** in 150 mL of dioxane was added and the mixture stirred in an ice–water bath for 15 min. The sodium salt which separated was filtered, washed with several portions of ether, suspended in water, and acidified with concentrated HCl to give the free acid (17 g, 92%). Because it was difficult to remove a persistent impurity, presumably CLIMOC-Phe-Phe-OH, a sample (2.5 g) of the crude product was purified on a Waters Prep-500 HPLC instrument using a C_{18} silica gel cartridge. Isocratic elution via MeOH–H_2O–HOAc (70:29:1) gave the pure amino acid, which was collected in a volume of 2 L (flow rate 100 mL/min). Rotary evaporation gave 2.38 g (95% recovery) of the acid, which after recyrstallization from ethanol was obtained as the **ethanol solvate**: mp 113–5 °C (softening at 75 °C); ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3, CH_3), 3.1 (dd, 2, $CH_2C_6H_5$), 3.5 (s, 2, CH_2CCl), 3.65 (q, 2, CH_2OH), 4.65 (br s, 1, CH), 5.1 (s, 2, CH_2OCO), 6.7–7.5 (m, 9, aryl).

Anal. Calcd for $C_{22}H_{24}ClNO_5$: C, 63.23; H, 5.79; Cl, 8.48; N, 3.35. Found: C, 63.24; H, 5.67; Cl, 8.56; N, 3.38.

Use of CLIMOC-OSu **12** for the acylation followed by recrystallization from $CHCl_3$–hexane gave, without the need for chromatography, the pure unsolvated amino acid, mp 126–8 °C.

Anal. Calcd for $C_{20}H_{18}ClNO_4$: C, 64.60; H, 4.85; Cl, 9.56; N, 3.77. Found: C, 64.53; H, 4.89; Cl, 9.55; N, 3.71.

***N*-(2-Chloroinden-3-ylmethyloxycarbonyl)glycine** was prepared as described for the phenylalanine analogue in 94% yield: mp 124–6 °C (anhydrous) or 86 °C (monohydrate); ^{1}H NMR ($CDCl_3$) δ 3.5 (s, 2, CH_2), 3.9 (d, 2, CH_2N), 5.1 (s, 2, CH_2O), 5.75 (br s, 1, NH), 7.3 (m, 4, aryl).

Anal. Calcd for $C_{13}H_{12}ClNO_4 \cdot H_2O$: C, 52.11; H, 4.67; Cl, 11.85; N, 4.67. Found: C, 52.05; H, 4.73; Cl, 11.50; N, 4.64.

***N*-(2-Chloroinden-3-ylmethyloxycarbonyl)glycyl Chloride.** A suspension of 2.8 g of CLIMOC-Gly-OH in 10 mL of $CHCl_3$ was treated with 3 mL of $SOCl_2$. The mixture was refluxed for 10 min, during which time the acid dissolved. The reaction mixture was cooled, filtered, and treated with 100 mL of hexane, to precipitate 2.3 g (77%) of the acid chloride: mp 75 °C dec; ^{1}H NMR ($CDCl_3$) δ 3.5 (s, 2, CH_2), 4.25 (d, 2, CH_2N), 5.15 (s, 2, CH_2O), 5.7 (br s, 1, NH), 7.15–7.5 (m, 4, aryl).

Anal. Calcd for $C_{13}H_{11}Cl_2NO_3$: C, 52.02; H, 3.69; N, 4.67. Found: C, 52.27; H, 3.62; N, 4.72.

3-Nitro-4-methoxybenzenesulfonyl Chloride. A solution of 50 g of 4-methoxybenzenesulfonyl chloride in 170 mL of concentrated H_2SO_4 was cooled in an ice–salt bath with vigorous mechanical stirring. Concentrated HNO_3 was added slowly with stirring such that the temperature remained near 0 °C. After the addition was complete, the solution was poured over ice diluted with 200 mL of ether. The layers were separated, and the aqueous layer was extracted with four 100-mL portions of ether. The combined extracts were dried (Na_2SO_4) and filtered, the drying agent was washed thoroughly with ether, and the solution was evaporated to give 48.9 g (80%) of the sulfonyl chloride as a tacky yellow solid. Recrystallization from Et_2O–hexane gave 34.3 g (56.3%) of the pure chloride in three crops as yellow crystals: mp 62–4 °C (lit.[26] mp 66 °C); ^{1}H NMR ($CDCl_3$) δ 4.25 (s, 3, OCH_3), 7.5 (d, 1, $aryl_1$), 8.3 (q, 1, $aryl_2$), 8.5 (d, 1, $aryl_3$).

3-Nitro-4-hydroxybenzenesulfonylated Polystyrene 21. A batch of polystyrene beads (40 g, XE-305, Rohm and Haas) was washed with warm dioxane (5 × 300 mL) and filtered until a drop of the filtrate spotted on a TLC plate no longer absorbed UV light. The beads were then washed with MeOH (2 × 300 mL), filtered, and dried thoroughly under vacuum in a rotary evaporator using vigorous steam heating. The dried beads were finely mixed with 3-nitro-4-methoxybenzenesulfonyl chloride (120 g, 0.48 mol) in a 1-L round-bottomed flask, and a mixture of $AlCl_3$ (68 g, 0.51 mol) in pure nitrobenzene (240 mL) was added. The flask was affixed with wire to a rotary evaporator in order to effect even mixing by rotation while the mixture was heated at atmospheric pressure in an oil bath to 85 °C. After 5 h of heating and rotation, the mixture was filtered and the beads were poured into a solution of DMF (150 mL), concentrated HCl (100 mL), and ice (150 g). The beads gradually lightened in color and after 45 min were filtered and washed with a solution of 50% DMF in H_2O until the washings were nearly colorless (ca. 5 × 300 mL). Finally the beads were washed with hot (110 °C) DMF (4 × 300 mL) and 70% CH_2Cl_2 in MeOH (4 × 300 mL) and dried in the rotary evaporator.

To effect hydrolysis of the methoxy function, a solution of 40% benzyltrimethylammonium hydroxide in H_2O (Triton B, 130 mL), DMSO (260 mL), and H_2O (130 mL) was added to the flask and the mixture was rotated on the rotary evaporator as before for 8 h at 100 °C. The polymer was filtered and the process repeated for another 8 h by using the same amounts of fresh Triton B–DMSO–H_2O. The beads were filtered and washed with copious amounts of warm water in 300-mL portions (ca. 4 L). The polymer was washed with warm dioxane (3 × 300 mL), filtered, and stirred with a solution of HOAc (40 mL) and dioxane (260 mL) for 15 min. Washing with dioxane was then continued until the washings were neutral, and the polymer was finally washed with 70% CH_2Cl_2 in MeOH (6 × 300 mL). The beads were filtered and dried thoroughly under vacuum in the rotary evaporator using steam heat. The derivatized polymer weighed 61.5 g. Elemental analysis (5.26% S; 2.56% N) indicated a loading of 1.6 or 1.8 mmol/g, respectively. The effective loading was determined in the following way:

A portion of the derivatized polymer (1.02 g) was rotated with benzoyl chloride (0.69 g, 0.57 mL, 4.92 mmol), pyridine (0.40 g, 0.40 mL, 4.92 mmol), and dry $CHCl_3$ (10 mL) for 30 min at 0 °C. The polymer was filtered, washed with $CHCl_3$ (3 × 25 mL), and added to a solution of benzylamine (0.27 g, 0.28 mL, 2.48 mmol) in $CHCl_3$ (10 mL). The mixture was rotated for 20 min at room temperture, and the polymer was filtered. Excess benzylamine was extracted with 5% HCl (2 × 10 mL), and evaporation of the solvent under vacuum afforded 0.338 g (1.6 mmol) of pure *N*-benzylbenzamide, thereby demonstrating the effective loading of the polymer to be 1.6 mmol of active OH groups per gram.

***N*-Benzyl-*N'*-butylcarbodiimide.** This compound has recently been described by Jaszay et al.[27] Our synthesis was patterned after methods B and C of Palomo and Mestres.[28] In our hands the method of Tartar and Gesquiere[29] was not suitable. To a stirred solution of 20.6 g of triphenylphosphine in 120 mL of CH_2Cl_2 at 0 °C was slowly added 12.5 g of bromine followed by 22.8 mL of triethylamine. The mixture was warmed to room temperature and treated portionwise over a period of 30 min with 10 g of *N*-benzyl-*N'*-butylurea (mp 100–1 °C). The resulting reaction mixture was stirred at room temperature for an additional 30 min and washed twice with 100-mL portions of water. The organic phase was separated and dried over $MgSO_4$, and the solvent was removed from a water bath (40 °C) with a rotary evaporator (10 mm) to afford a brown solid, which was extracted four times with 60-mL portions of hexane. The combined hexane extracts were evaporated to give 8.90 g of brown oil, which was dissolved in 30 mL of pentane. The resulting pentane solution was cooled in the freezer and filtered while cold to remove a small amount of crystalline material, which was identified as triphenylphosphine oxide. The filtrate was concentrated from a water bath (25 °C) with a rotary evaporator (10 mm) and the residue distilled from an oil bath (120 °C) through a simple Claisen head to give 5.02 g (55%) of the carbodiimide as a colorless liquid: bp 90–2 °C (0.8–1 mm) [lit.[27] bp 88–9 °C (0.15 mm)]; ^{1}H NMR ($CDCl_3$) δ 0.65–1.60 (m, 7, $CH_3CH_2CH_2$), 3.10 (t, 2, NCH_2CH_2), 4.30 (s, 2, $ArCH_2$), 7.30 (m, 5, aryl).

CLIMOC-Phe-4-Hydroxy-3-nitrobenzophenone and the Corresponding Sulfone Polymeric Esters. To a solution of 740 mg of CLIMOC-Phe-OH in 7 mL of THF was added 1 g of a polymer bearing 1.7 mmol of 4-hydroxy-3-nitrobenzophenone functions per gram. The mixture was cooled to −10 °C and 400 mg of benzyl-*n*-butylcarbodiimide added. After the mixture was rotated at a temperature not exceeding −5 °C for 3 h, the polymer

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was washed in succession with dry, cooled THF (4 × 15 mL), CH_2Cl_2 (3 × 15 mL), and ether and finally dried by passage of a slow stream of air. The final polymer held 0.55 mmol/g of protected phenylalanine residues as calculated from its chlorine analysis (1.95% Cl). Similarly the polymeric active ester of CLIMOC-Gly-OH was obtained (0.52 mmol/g). Similar methods were used to load the more reactive 3-nitro-4-hydroxybenzenesulfonylated polymer **21** having an effective loading of 0.71 mmol of OH/g with the following amino acids leading to the loading indicated: CLIMOC-Phe-OH (0.47 mmol/g), CLIMOC-Gly-OH (0.52 mmol/g), and FMOC-Tyr(Bn)-OH (0.36 mmol/g).

CLIMOC-Gly-Phe-Leu-OBn. Leucine benzyl ester *p*-toluenesulfonate (600 mg) was neutralized with excess $NaHCO_3$ and the solution extracted into 35 mL of CH_2Cl_2. After drying ($MgSO_4$), 6 g of polymer **22** (R = $C_6H_5CH_2$, X = CLIMOC; 0.47 mmol/g) was added to the CH_2Cl_2 solution and the mixture was rotated for 30 min. After filtration, the polymer was extracted with eight 15-mL portions of CH_2Cl_2 and the combined filtrate and washings were evaporated to give 820 mg (95%) of the dipeptide CLIMOC-Phe-Leu-OBn, mp 131–2 °C. Without any purification, the dipeptide was deblocked by dissolving it in $CHCl_3$ and rotating it for 1.5 h with 8 g of piperazino silica reagent **6** loaded with 1 mmol of NH per gram. The silica gel reagent was washed with six 100-mL portions of CH_2Cl_2, and the combined washings were evaporated to give 495 mg (90%) of the free amino peptide. This was in turn dissolved in 30 mL of CH_2Cl_2 and treated as described above with 5 g of polymer **22** (R = H, X = CLIMOC; 0.52 mmol/g) for 1 h. Workup as given above gave 740 mg (91%) of the protected tripeptide: mp 123–4 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.86 (d, 6, Me_2C), 1.55 (m, 3, CH_2CH), 3.1 (m, 2, β-CH_2, Phe), 3.6 (s, 2, CH_2CCl), 3.84 (d, 2, CH_2N), 4.6 (m, 2, NCHCO), 5.15 (s, 2, CH_2O), 5.18 (s, 2, CH_2O), 5.45 (t, 1, NH, Gly), 6.38 (d, 1, NH), 6.72 (d, 1, NH), 7.2–7.38 (m, aryl).

Anal. Calcd for $C_{35}H_{38}ClN_3O_6$: C, 66.50; H, 6.06; Cl, 5.61; N, 6.64. Found: C, 66.79; H, 6.32; Cl, 5.41; N, 7.07.

CLIMOC-Gly-Gly-Phe-Leu-OBn. In a separate experiment, the synthesis as described above was continued to the tetrapeptide stage without stopping at any point. The tetrapeptide was obtained in 74% yield: mp 127–8 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.83 (d, 6, Me_2C), 1.5 (m, 3, CH_2CH), 3.1 (m, 2, β-CH_2, Phe), 3.53 (s, 2, CH_2CCl), 3.96 (br s, 4, CH_2N), 4.58 (m, 1, NCHCO), 4.9 (m, 1, NCHCO), 5.09 (s, 2, 5-ring CH_2O), 5.16 (s, 2, $C_6H_5CH_2O$), 5.88 (t, 1, NH, Gly), 7.0 (d, 1, NH), 7.08–7.26 (m, aryl, NH).

Anal. Calcd for $C_{37}H_{41}ClN_4O_7$: C, 64.48; H, 6.00; Cl, 5.15; N, 8.13. Found: C, 64.51; H, 6.16; Cl, 5.09; N, 7.95.

Benz[*f*]indene-1-methanol (15). A 1.0 M solution of *n*-butyllithium (44 mL, 44 mmol) was added dropwise under a nitrogen atmosphere to a stirred solution of 5 g (30.12 mmol) of benz[*f*]indene (**14**) in 140 mL of anhydrous ether and 20 mL of anhydrous THF cooled by means of a dry ice–acetone bath to −70 °C. The temperature of the reaction mixture was not allowed to exceed −50 °C. Benz[*f*]indenyllithium soon started to precipitate as small red crystals. After completion of the addition (about 2 h), the reaction mixture was stirred at −70 °C for another 45 min before introduction of formaldehyde. Paraformaldehyde, 13.5 g, dried overnight in vacuum over phosphorus pentoxide, was stirred and heated in a dry flask placed in an oil bath at 175–95 °C. The formaldehyde gas was led through a 7-mm glass tube into the benz[*f*]indenyllithium solution (held below −50 °C) by a stream of dry nitrogen. The temperature was not allowed to exceed −50 °C. After completion of the addition, 280 mL of 10% HCl solution was slowly poured into the stirred reaction mixture. The mixture was stirred for 15 min at room temperature. After the ether layer was separated, the aqueous solution was extracted twice with 50-mL portions of ether, and the combined ether solution was washed with small portions of water until neutral. The ether solution was dried ($MgSO_4$) and evaporated to give a light brown oil (6 g). Storage in the freezer gave a soft yellow solid, which was purified by chromatography (100 g of silica gel, 1:1 ethyl acetate–hexane) to give 3.5 g (59%) of the alcohol as a yellow solid. The NMR showed the solid to be a mixture of benz[*f*]indene-1-methanol (**15**) (>95%) and benz[*f*]indene-3-methanol (**16**). Several recrystallizations from ligroin (bp 88–9 °C) gave 3.0 g (50.6%) of pure, colorless benz[*f*]indene-1-methanol (**15**): mp 115–6 °C; ^{1}H NMR ($CDCl_3$) δ 1.50–1.70 (br, 1, OH), 3.75–4.00 (m, 3, $CHCH_2$), 6.45–6.65 (m, 1, H_2), 6.85–7.05 (m, 1, H_3), 7.25–8.00 (m, 6, aryl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.5 (ring CH_2), 64.3 (CH_2O), 119, 122, 125.4, 125.8, 128, 132, 133, 133.5, 137.5, 142.2, 142.3, 143.5 (vinyl and aryl); IR (KBr) 3500–2400 cm^{-1} (OH).

Anal. Calcd for $C_{14}H_{12}O$: C, 85.68; H, 6.16. Found: C, 85.40; H, 6.20.

Benz[*f*]indene-3-methanol (16). A solution of 3.5 g of crude benz[*f*]indene-1-methanol (**15**), 1.33 mL of triethylamine, and 13.3 mL of pyridine was stirred at room temperature for 21 h. The stirred reaction mixture was cooled by means of an ice bath and treated slowly with 90 mL of 10% HCl solution. The resulting suspension was stirred at room temperature for 10 min and extracted with three 50-mL portions of ether. The ether solution was washed three times with 50-mL portions of water, dried ($MgSO_4$), and evaporated to give 2.6 g (44.1% from benz[*f*]indene) of the crude alcohol as a yellow solid. Recrystallization from ligroin (bp 88–90 °C) gave 2.2 g (37.3%, 65.7% recovery) of the pure alcohol **16** as colorless crystals: mp 123.5–124.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.65 (br, 1, OH), 3.45 (m, 2, $ArCH_2$), 4.75 (m, 2, CH_2O), 6.55 (m, 1, CH=), 7.30–7.95 (m, 6, aryl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37 (ring CH_2), 60 (CH_2O), 117, 122.3, 125, 125.3, 127.9, 128.3, 131.2, 132, 133, 142.4, 142.5, 143.7 (vinyl and aryl); IR (KBr) 3500–2400 cm^{-1} (OH).

Anal. Calcd for $C_{14}H_{12}O$: C, 85.68; H, 6.16. Found: C, 85.87; H, 6.27.

Benz[*f*]inden-3-ylmethyl Chloroformate (17). To an ice-cold, stirred solution of 7 mL of phosgene in 10 mL of dry THF was added dropwise a solution of 2.5 g of benz[*f*]indene-3-methanol (**16**) in 25 mL of dry THF within 1 h. The reaction mixture was allowed to stand at 0 °C for another 2.5 h. The solvent and excess phosgene were removed from a water bath (25 °C) with a water aspirator (10 mm). In order to remove traces of phosgene, 25-mL portions of pentane were added and the solution was evaporated three times. Eventually 3.1 g (94%) of the crude chloroformate was obtained as a yellow solid. Recrystallization twice from ether gave 2 g (61%) of the pure chloroformate as colorless crystals: mp 56–7 °C; IR (KBr) 1770 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 3.5 (m, 2, $ArCH_2$), 5.35 (m, 2, CH_2O), 6.70 (m, 1, CH=), 7.35–8.00 (m, 6, aryl).

Anal. Calcd for $C_{15}H_{11}ClO_2$: C, 69.64; H, 4.28. Found: C, 69.43; H, 4.48.

Benz[*f*]inden-3-ylmethyl Azidoformate (18). To an ice-cold, stirred solution of 0.16 g of NaN_3 in 1.1 mL of H_2O was added slowly a solution of 0.42 g of chloroformate **17** in 0.9 mL of acetone. The mixture was stirred in an ice bath for 2 h and at room temperature for 2 h. The reaction mixture was extracted twice with 20-mL portions of ether. The combined ether extracts were washed three times with 10-mL portions of H_2O and dried ($MgSO_4$). Removal of solvent from a water bath (30 °C) with a rotary evaporator (7 mm) gave 0.46 g of the crude azide, mp 49–53 °C. Recrystallization twice from hexane gave 0.33 g (81%) of the azide as colorless crystals: mp 58–9 °C; IR (KBr) 2159 (N_3), 1720 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 3.5 (m, 2, $ArCH_2$), 5.3 (s, 2, CH_2O), 6.65 (m, 1, CH=), 7.25–8.1 (m, 6, aryl).

Anal. Calcd for $C_{15}H_{11}N_3O_2$: C, 67.92; H, 4.18; N, 15.84. Found: C, 68.15; H, 4.30; N, 16.00.

***N*-(Benz[*f*]inden-3-ylmethyloxycarbonyl)phenylalanine.** To an ice-cold, stirred solution of 0.200 g of azidoformate **18** in 7.5 mL of THF was added very slowly an ice-cold solution of 0.105 g of phenylalanine in 3 mL of 10% aqueous Na_2CO_3 solution. The reaction mixture was stirred at 0 °C for 96 h and then treated with 20 mL of water. The aqueous solution was extracted three times with 10-mL portions of ether, cooled in an ice bath, and acidified with 10% aqueous HCl solution to Congo red. The resulting white precipitate (220 mg, 76%), mp 176–7 °C, was collected by suction filtration. Recrystallization from MeOH–H_2O (3:1) gave 205 mg (70.2%) of pale yellow crystals, mp 191.5–2.5 °C, believed to be a methanol complex of the protected acid: IR (KBr) 3300 (NH and OH), 1770 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 2.95–3.25 (m, 3, $ArCH_2$ and NCH), 3.45 (m, 2, ring CH_2), 3.55 (br, 3, CH_3O), 4.45 (m, 1, NH), 5.15 (m, 2, CH_2O), 6.55 (m, 1, CH=), 7.10–7.95 (m, 11, aryl).

Anal. Calcd for $C_{25}H_{25}NO_5$: C, 71.58; H, 6.00; N, 3.34. Found: C, 71.95; H, 5.55; N, 3.75.

***N*-(Benz[*f*]inden-3-ylmethyloxycarbonyl)glycine**: obtained from BIMOC-Cl and glycine (room temperature, 1.5 h) in 90% yield, mp (crude) 147–50 °C, or from BIMOC-N_3 as

described for phenylalanine (0 °C, 72 h) in 89% yield, mp (crude) 146–9 °C. Samples obtained from BIMOC-Cl were contaminated by varying amounts of BIMOC-Gly-Gly-OH (HPLC) which could not be removed by recrystallization. Recrystallization of the samle derived from BIMOC-N_3 from MeOH–H_2O gave the pure acid with 92% recovery: mp 152.5–3.5 °C; IR (KBr) 3400 (NH and OH), 1760, 1670 cm^{-1} (C=O); ^1H NMR (DMSO-d_6–$CDCl_3$) δ 3.55 (m, 2, CH_2Ar), 3.85 (d, 2, NCH_2CO), 5.25 (m, 2, CH_2O), 6.65 (m, 1, CH=), 6.85 (t, 1, NH), 7.40–7.95 (m, 6, aryl).

Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.69; H, 5.05; N, 4.71. Found: C, 68.31; H, 5.05; N, 4.79.

***N*-(Benz[*f*]inden-3-ylmethyloxycarbonyl)glycylglycine.** To a solution of 0.26 g of chloroformate **17** in 8 mL of dioxane was added with stirring and cooling in an ice bath a solution of 0.135 g of glycylglycine in 3.22 mL of 10% Na_2CO_3. The mixture was stirred at room temperature for 8 h and worked up as given for the phenylalanine derivative. The crude product was obtained as a white solid (87.5%; mp 203–4.5 °C), which upon recrystallization from methanol gave the pure dipeptide (80%) as colorless crystals: mp 210–1 °C; IR (KBr) 3350, 3330 (NH and OH), 1720, 1670 cm^{-1} (C=O); ^1H NMR (DMSO-d_6) δ 3.50 (m, 2, CH_2Ar), 3.70 (m, 4, CH_2CO), 5.10 (m, 2, CH_2O), 6.65 (m, 1, CH=), 7.30–8.20 (m, 6, aryl).

Anal. Calcd for $C_{19}H_{18}N_2O_5$: C, 64.41; H, 5.08; N, 7.91. Found: C, 64.27; H, 5.25; N, 7.73.

Loading of BIMOC-Protected Amino Acids onto 4-Hydroxy-3-nitrobenzenesulfonylated Polystyrene. A sample of the phenolic polymer bearing 1.78 mequiv of OH/g was loaded in the usual manner with BIMOC-Phe-OH, BIMOC-Gly-OH, and FMOC-Tyr(Bn)-OH to provide active esters with effective loadings of 0.59, 0.58, and 1.10 mmol/g of protected amino acid, respectively. The following is a representative example:

To a solution of 0.865 g of FMOC-Tyr(Bn)OH in 30 mL of dry THF was added 2.40 g of 3-nitro-4-hydroxybenzenesulfonylated polystyrene **21**. The resulting mixture was cooled at 0 °C by means of an ice bath and treated with 0.66 g of *N*-benzyl-*N'*-butylcarbodiimide. The mixture was rotated as noted for the CLI-MOC-phenylalanine derivative at 0 °C for 4.5 h. The polymer was filtered and washed in sequence with eight 20-mL portions each of THF, CH_2Cl_2, and ethyl ether. Air drying gave 3.06 g of polymer as yellow beads, which by elemental analysis for nitrogen (3.50%) showed a loading of 1.10 mequiv of protected amino acid per gram of polymer.

BIMOC-Phe-Leu-OBn. Leucine benzyl ester *p*-toluenesulfonate (236 mg, 0.6 mmol) was treated with 10 mL of 5% aqueous $NaHCO_3$ solution with stirring for 15 min, and the mixture was extracted with 15 mL of CH_2Cl_2. The CH_2Cl_2 solution was dried over anhydrous $MgSO_4$ and treated with 1.5 g of the polymeric ester of BIMOC-Phe **22** (R = $C_6H_5CH_2$, X = BIMOC; 0.852 mmol). The reaction mixture was rotated on a rotary evaporator shaft at room temperature for 50 min (TLC analysis showed that the reaction was complete within 30 min), and the spent polymer was filtered and washed eight times with 10-mL portions of methylene dichloride. The combined washings were evaporated from a water bath (25 °C) with a rotary evaporator (10 mm) to afford 300 mg (85%) of the ester as a pale yellow solid, mp 109–10 °C. TLC and HPLC analysis showed that the crude product showed essentially no impurities. After recrystallization from 10 mL of ethyl acetate–hexane (1.5:8.5), 270 mg (77%) of the protected dipeptide was obtained as pale yellow crystals: mp 110.5–1.5 °C; $[\alpha]^{24.5}_D$ = –6.0 (*c* 0.3, ethyl acetate); HPLC [methanol, *f* = 2, Waters 0.8 × 10 cm, C-18, 10 μ column (Radial-PAK)], t_R = 2.35 min (100%); IR (KBr) 3300 (NH), 1735, 1690, 1650 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 0.85 [m, 6, $(CH_3)_2$], 1.2–1.6 (m, 3, CH_2CH), 3.05 (m, 2, $C_6H_5CH_2$), 3.5 (m, 2, ring CH_2), 4.40–4.65 (m, 2, NCHCO), 5.1 (m, 2, OCH_2C=C), 5.2 (s, 2, $OCH_2C_6H_5$), 5.35 (br, 1, NH), 6.1 (br, 1, NH), 6.55 (m, 1, CH=), 7.1–7.9 (m, 16, aryl).

Anal. Calcd for $C_{37}H_{38}N_2O_5$: C, 75.23; H, 6.48; N, 4.74. Found: C, 75.22; H, 6.51; N, 4.59.

Silica Gel Supported 4-(Aminomethyl)piperidine (23). A solution of 30 mL of (3-chloropropyl)trimethoxysilane and 90 mL of 4-(aminomethyl)piperidine in 600 mL of toluene was refluxed for 2 h. The resulting suspension was cooled to room temperature and filtered to remove the byproduct hydrochloride. The filtrate was concentrated from a water bath (70–80 °C) with a rotary evaporator (10 mm) to give a colorless oil, which was distilled from an oil bath at 140 °C to give again a colorless oil (12 g, 26.7%), bp 107–10 °C (0.035 mm). The mixture of silylamines **24** and **25** [^1H NMR ($CDCl_3$) δ 0.6 (m, 2, $SiCH_2$), 0.95–1.90 (m, 8, NH and CCH_2C), 2.25–2.6 (m, 6, NCH_2), 2.9 (m, 2, NCH_2), 3.05 (m, 2, NCH_2), 3.4 (m, 1, NH), 3.55 (m, 9, Me)] was used as such for conversion to the silica-based deblocking agent. To a mixture of **24** and **25** (14.7 g) in 120 mL of toluene was added 29 g of silica gel (60–200 mesh). The mixture was stirred under N_2 for 1.5 h at room temperature and then heated to reflux and distilled to remove 25 mL of a mixture of methanol and toluene. After a further 1 h of refluxing, an additional 25 mL of methanol–toluene was distilled out. Finally, the mixture was refluxed for another 45 min, cooled, and filtered and the silica gel washed several times with toluene, followed by hexane. After drying in a desiccator (5 mm) overnight at room temperture, there was obtained 36.70 g (95%) of the functionalized silica gel. Elemental analysis for nitrogen (2.80%) showed a loading of 1.0 mequiv of NH/g of polymer.

Two-Support Manual Synthesis of FMOC-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn via BIMOC Protection. In this synthesis, each acylation step involved rotation of the polymeric active ester **22** with the amino acid ester or peptide ester from a previous step in DMF solution for about 1 h (TLC showed complete reaction had occurred after 30 min). At the end of this time, the polymer was filtered and washed with eight 15-mL portions of CH_2Cl_2. The filtrate and washings were evaporated at 25 °C (10 mm) to bring the volume back to that of the original DMF solution. Each deblocking step was carried out for 3.5 h by using 11 g of piperidino silica gel **23** containing 1 mmol of active reagent per gram. Filtration was followed by eight 15-mL washings with CH_2Cl_2 and evaporation as before. The synthesis was initiated with 132 mg (0.6 mmol) of H-Leu-OBn, 30 mL of DMF, and 2 g (1.14 mmol) of BIMOC-Phe-Ⓟ. In each case, 2 g of the individual polymeric esters [BIMOC-Phe-O-Ⓟ, BIMOC-Gly-O-Ⓟ (twice), and FMOC-Tyr(Bn)-O-Ⓟ] was used. At the end of the synthesis, the FMOC pentapeptide was chromatographed on silica gel (50 g; 200–400 mesh) by using EtOAc–hexane–HOAc (64:32:4) as eluent to give, after crystallization that THF–hexane, 195.8 mg (34%) of the peptide ester as a white solid; mp 172–6 °C (lit.[20] mp 178 °C); $[\alpha]^{22}_D$ = –16.1 (*c* 0.5, DMF) [lit.[20] $[\alpha]^{25}_D$ = –16.9 (*c* 0.9, DMF)]; ^1H NMR ($CDCl_3$) δ 0.75 (m, 6, CH_3), 1.4–1.6 (m, 3, CH_2CH), 2.85–3.10 (d, 4, CH_2 (Tyr and Phe)], 4.05 [m, 1, α-H(Tyr)], 4.10 (m, 4, α-H(Gly_{2+3}), 4.20 (m, H, FlCH), 4.35 (d, 2, CH_2O), 4.65 [m, 1, α-H(Leu)], 4.70 [m, 1, α-H(Phe)], 4.8 (s, 2, $OCH_2C_6H_5$), 4.9 (s, 2, $OCH_2C_6H_5$), 5.0 (d, 1, NH), 5.1 (br, 2, NH), 6.65 (br, 1, NH), 6.75–7.80 (m, 27, aryl), 7.85 (br, 1, NH); identified by comparison of NMR, TLC, and HPLC data with those of an authentic sample. It is believed that the low yield obtained in this initial run in DMF was caused by inefficient washing of the polymeric reagents by CH_2Cl_2. In this run, the first deblocking step was allowed to proceed by mistake for 11 h in spite of the fact that TLC evidence indicated complete deblocking in all other deblocking steps after 3.5 h. This was attributed to incomplete removal of CH_2Cl_2 following the first acylation step since deblocking is slower in a mixture of DMF and CH_2Cl_2 than in DMF alone.

[Leu]enkephalin. To a stirred mixture of 77 mg of 10% Pd–C in 8 mL of MeOH–dioxane (1:1) were added 77 mg of $Pd(OAc)_2$ and 200 mg of NH_4OCHO. After 1 min, 77 mg of FMOC pentapeptide ester **27** was added and the reaction mixture stirred at room temperature for 1 h. The catalyst was filtered and washed several times with small portions of ether. The combined organic layers were evaporated from a water bath (25 °C) with a rotary evaporator (10 mm) to give a colorless oil, which was dissolved in MeOH and treated with ether to give 34 mg (73.6%) (two crops) of the free peptide as a white solid: mp 160–2.5 °C (lit.[20] mp 158 °C; lit.[30] mp 206 °C); $[\alpha]^{23}_D$ = –37.1 (*c* 0.5, DMF) [lit.[31] $[\alpha]^{23}_D$ = –26.1 (*c* 1, DMF)]; TLC, one spot (identical with that shown by an authentic sample[20]); ^1H NMR (DMSO-d_6) δ 0.9 [m, 6, $(CH_3)_2$], 1.45–1.70 (m, 3, CH_2CH), 2.60, 2.80, 3.05 [m, 4, CH_2(Tyr and Phe)], 3.55 [m, 1, α-H(Tyr)], 3.70 [d, 4, α-H(Gly_{2+3})], 4.1 [m, 1, α-H(Leu)], 4.45 [m, 1, α-H(Phe)], 6.7, 7.0 [d, 4, aryl(Tyr)], 7.2 [m, 1, NH(Tyr)],

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7.25 [m, 5, aryl(Phe)], 7.95 [m, 2, NH(Gly_3 and Leu)], 8.2 [d, 1, NH(Phe)], 8.45 [br, 1, NH(Gly_2)]. The NMR spectrum was superimposable on that published by Garbay-Jaureguiberry and co-workers.[32]

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Registry No. **7**, 95-13-6; **9**, 18427-72-0; **10**, 88099-20-1; **11**, 88099-21-2; **12**, 123150-47-0; **14**, 268-40-6; **15**, 123150-49-2; **16**, 123150-50-5; **17**, 123150-51-6; **18**, 123150-52-7; **24**, 123150-57-2; **25**, 123150-58-3; **26**, 88099-27-8; **27**, 88099-29-0; HONSu·DCHA, 82911-72-6; H-Phe-OH, 63-91-2; CLIMOC-Phe-OH, 88099-22-3; CLIMOC-Phe-Phe-OH, 123150-48-1; H-Gly-OH, 56-40-6; CLIMOC-Gly-OH, 88099-23-4; CLIMOC-Gly-Cl, 88099-24-5; 4-$MeOC_6H_4SO_2Cl$, 98-68-0; 3-(O_2N)-4-$(MeO)C_6H_3SO_2Cl$, 22117-79-9; BnNHCONHBu, 14117-22-7; BnN=C=NBu, 111681-30-2; H-Leu-OBn·TsOH, 1738-77-8; CLIMOC-Phe-Leu-OBn, 88099-25-6; BIMOC-Phe-OH, 123150-53-8; BIMOC-Gly-OH, 123150-54-9; H-Gly-Gly-OH, 556-50-3; BIMOC-Gly-Gly-OH, 123150-55-0; FMOC-Tyr(Bn)-OH, 71989-40-7; BIMOC-Phe-Leu-OBn, 123150-56-1; $(MeO)_2Si(CH_2)_3Cl$, 2530-87-2; H-Leu-OBn, 1738-69-8; BzCl, 98-88-4; $BnNH_2$, 100-46-9; BzNHBn, 1485-70-7; 4-(aminomethyl)piperidine, 7144-05-0; Leu-enkephalin, 58822-25-6; polystyrene, 9003-53-6.

Crystallographic Studies on Retinoidal-Active and -Inactive Aromatic Anilides

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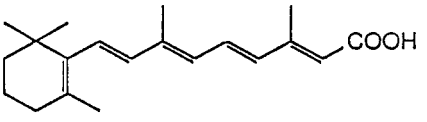
Among various chemical classes of compounds with retinoidal activity, the retinobenzoic acid system having a 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl moiety and a benzoic acid moiety at opposite ends of the molecule has proved to be one of the most promising leads. Many retinoidal-active compounds with various kinds of chemical moieties as the linking group between the above two groups were synthesized, and amide moieties proved to be excellent linkages for potent retinoidal activities. The activities, however, were diminished by methylation at the amide nitrogen. To elucidate the reasons for the loss of the activities, we performed X-ray crystal structure analyses of three free amides and the corresponding three *N*-methylamide compounds. It was proved that all the free amide molecules take an extended trans conformation, whereas all the *N*-methylamide molecules take a folded cis conformation in the crystalline state. In combination with spectroscopic studies (UV and NMR), the above results suggested that these *N*-methylamides adopt remarkable folded conformations compared to the free amide molecules in solution. These facts strongly indicate that all these *N*-methylamides take cis conformations not only in the crystal but also in solution. It is the extended trans conformation that is required for specific binding to the retinoidal receptor macromolecule. Consequently, the loss of activities in *N*-methylamides seem to be ascribed to conformational factors but not to steric hindrance by the methyl group which might prevent binding to the target receptor.

Introduction

Retinoic acid has attracted much interest because of its wide-ranging biological activities.[1] A vast number of related compounds have been synthesized and assayed for retinoidal activity so far. From the structure–activity relationship studies on these synthetic compounds, it is suggested that the long polyene chain in natural retinoic acid (1) is not essential for the retinoidal activities, but a



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carboxylic acid at one end as well as a bulky hydrophobic group at the other end of a long molecule are essential. Furthermore, the permissible structures of the internal group (so-called linkage group) inserted between these two groups are surprisingly wide-ranging, even in compounds with extremely potent activities. This fact seems to show that the linkage group does not play an important role in specific binding to the retinoidal receptor but regulates the positional and conformational relations between the two groups. After many trials of skeletal conversion of retinoic acid, retinobenzoic acids were found, which are defined as a series of benzoic acid derivatives with potent retinoidal activities. Among various kinds of retinobenzoic acid, a structure with a 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl group and a benzoic acid group at opposite ends of the molecule proved to be a promising lead compound[2] showing potent retinoidal activities. Some of these compounds showed much higher activities than retinoic acid in inducing differentiation of human promyelocytic leukemia cells HL-60 and in other bioassays. The generic chemical structure of this series of compounds is illustrated in Figure 1, together with atomic numbering. The linkage

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