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## TOPOLOGICAL STEREOCHEMISTRY

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## INTRODUCTION

Consideration of molecular structures as graphs or framework models consisting of points (nuclei) connected by lines (bonds) is a ubiquitous and highly productive methodology in chemistry The geometrical properties of such models are, of course, central to our understanding of structure and reactivity In particular, chemists have long been intrigued by the underlying structural causes of isomerism, and framework models provide a very simple and satisfying explanation of this phenomenon Indeed, it was out of a necessity to explain the existence of constitutional isomers, those differing by bond connectivity, that the very concept of the connectivity of atoms was first reluctantly considered by organic chemists such as Kekule and Couper in the 1850s It was not until "examples of another kind of isomerism, 'optical isomerism', became too numerous to ignore" that the concept of the tetrahedral carbon atom was first proposed by van't Hoff and Le Bel [1]

Today, these concepts, chemical bonds between pairs of atoms (molecular constitution or *topology*) and the Euclidean geometry of molecules (stereochemistry) are taken for granted by most chemists In a theoretical sense, they may be considered separate That is, stereochemistry is not generally a consequence of the topology of a molecular structure alone, but rather derives from Euclidean geometrical properties of the structure There is a class of compounds, however, that stands at the topology–stereochemistry interface These are stereoisomers owing their distinct character solely to bond connectivity, requiring no Euclidean molecular rigidity at all to remain chemically different The term *topological stereoisomers* is proposed to describe these novel molecular entities *Topological stereochemistry* simply deals with the synthesis, characterization, and consideration of the chemically relevant geometry of topological stereoisomers [2] Such novel constructs as molecular links (catenanes), knots, and Möbius strips belong in the realm of topological stereochemistry While perhaps a relatively quiet backwater in the main stream of chemical research, topological stereochemistry is now recognized as an important aspect of molecular biology Indeed, topological stereochemistry is a unique field, esthetically and intellectually pleasing in the extreme The goal of this report is to allow the reader to appreciate fully the beauty of this interplay between molecular topology and stereochemistry

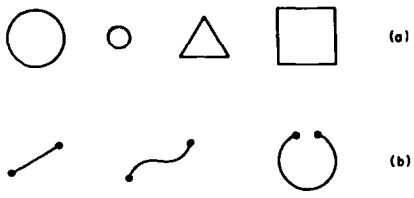
The report is divided into six parts Part I defines some basic topological ideas in terms synthetic chemists should find familiar, and puts forth a definition of topological stereochemistry, Parts II–IV deal with chemical synthesis of topological stereoisomers and related constructions, Part V describes an approach to specification of configuration for topological stereoisomers, and finally, Part VI presents a discussion of topological stereoisomerism of DNAs

## PART I. TOPOLOGY FROM A SYNTHETIC CHEMIST'S PERSPECTIVE

### A *Topology of one-dimensional constructions in three-dimensional space*

In order to appreciate topological stereochemistry, some "qualitative" knowledge of topology is required [3] Organic and inorganic chemists are, in fact, well prepared for a basic understanding of the required topology by virtue of their ability to manipulate molecular models and structural formulas One merely need define some topological and graph theoretical ideas in terms the chemist is already familiar with This is most easily accomplished by first considering some well-known Euclidean geometry

One-dimensional constructions are simply those composed of points and lines Consider the one-dimensional constructions shown in Scheme 1(a) Each is easily recognized as distinct from the others, and each has certain metric properties, i e properties that can be measured For example, the large circle has a radius, circumference, and area that can be measured It is clearly different from the small circle Similarly, the triangle and square have metric properties including angles, areas, and lengths of sides These objects may be moved about in the plane or in three-dimensional space (abbreviated 3-space), and these metric properties will remain the same They are said to be Euclidean invariants of the objects The distinct character of each of these constructions with respect to the others is, of course, a Euclidean invariant




Scheme 1 Some one-dimensional constructions

Topology for our purposes, more precisely low-dimensional topology, involves geometrical properties which remain invariant given continuous deformation in 3-space Such geometrical properties are called topological properties, or topological invariants To visualize the continuous deformation that preserves topological properties in 3-space, consider the construction to be totally flexible, as if it were made of infinitely stretchable rubber threads Lengths and angles are no longer invariant Indeed, no metric properties are topologically invariant But, connectivity is invariant, that is a line may not be broken, one line may not pass through another, and points may not become congruent Thus, the four constructions in Scheme 1(a) are all *topologically equivalent* That is, each may be deformed into the others by continuous deformation They are said to be *isotopic*, and they cannot be differentiated by consideration of their topology alone They owe their distinct character to Euclidean metric properties (rigidity) The circle, triangle, and square are thus simply different representations of the same topological construction—a closed curve Such representations are termed *presentations* of the construction

The four presentations of a closed curve shown in Scheme 1(a) also share another important topological property each has *identical connectivity* Connectivity used in this context has a very precise definition in topology But, the intuitive meaning of the phrase "identical connectivity" will serve for the present discussion Objects with identical connectivity are termed *homeomorphic* While the topological attributes isotopic and homeomorphic ascribed to pairs of objects have a similar meaning, they differ in a crucially important way In this report, the term "isotopic" is synonymous with "topologically equivalent", and "not isotopic" is synonymous with "topologically distinct" As discussed below, homeomorphic objects may be topologically equivalent or topologically distinct, but objects which are not homeomorphic must be topologically distinct

Consider now the objects in Scheme 1(b) Here are three presentations of a topological line Clearly, these presentations of the line are homeomorphic and isotopic They are, however, topologically distinct from the constructions shown in Scheme 1(a) The closed curve has no ends while the line has two ends The two-endedness of a line is a topological invariant It is independent of the length of the line, its shape or any metric properties Similarly, the lack of ends of a closed curve is also a topological invariant The constructions shown in Scheme 1(a) have different connectivity than those shown in Scheme 1(b), they are *not homeomorphic*, and the constructions in Scheme 1(a) cannot be converted to those in Scheme 1(b) by continuous deformation in 3-space, that is they are *not isotopic*

Unfortunately, because of ambiguity in accepted English definitions according to Webster,[4] topology may have another meaning quite opposed to that given above Topology is sometimes taken to mean "the study of the topography of a place" This is indeed unfortunate, since topography refers to the shape of a surface, a decidedly non-topological entity Since the topology of molecular structures is such a useful concept in chemistry, distinct from shape, we strongly recommend that when describing the shape or steric environment of, e g diastereotopic faces of a carbonyl group, chemists use the term *topography*, reserving the term topology for properties depending only on connectivity, as described above

B *The molecular graph*

The above discussion of the topology of one-dimensional constructions such as closed curves and lines is directly transferable to molecular structures once the *molecular graph* is defined A graph is simply any one-dimensional construction composed of points and lines [5] Lines joining points are called *edges* and points joined to more than two other points are termed *vertices* We define the molecular graph as simply the graph where nuclei define the points and bonds define the edges Also, different atomic nuclei, e g carbon and oxygen, define differentiated (differently colored) points Thus, the molecular graph is exactly the common structural formula embedded in 3-space, or a framework

molecular model Often, the hydrogen atoms are left off the molecular graph, though they are, or course, implied In this report, the name of a chemical entity is used to refer to the actual compound and to its molecular graph

As is the case for many definitions in stereochemistry, considerable arbitrariness is built into the definition of a molecular graph Thus, Mislow has pointed out that construction of a molecular graph, or what he terms the constitutional graph, is arbitrary since a yes or no decision must be made concerning whether two atoms are bonded or not He proposes the edge weighted complete graph, where each atom is considered bonded to every other atom in the structure, as a more accurate description of a molecule for the purposes of determining pairwise relationships between isomeric structures [6] In this graph, the edge weightings are values associated with the "degree" of bondedness of the atoms

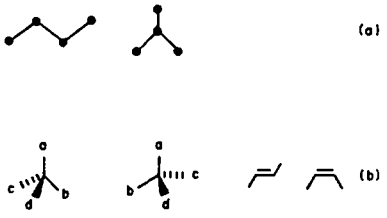
We define an edge of the molecular graph to be a covalent bond Thus, H-bonds, ion–ion bonds, ion–dipole bonds, or dipole–dipole bonds are not considered edges of a molecular graph A host–guest complex has identical topology with the uncomplexed host and guest by this definition But, a metal–porphyrin complex is topologically distinct from the separated porphyrin and metal atom Of course, even the term covalent bond is arbitrary Some metric bond strength must be assigned for a *topologically significant bond* Thus, the molecular graph is the Mislow complete edge weighted graph with all edges below a certain weighting removed Rather than make an arbitrary decision concerning the exact weighting necessary for a topologically significant bond, we choose to leave this point open For most compounds, chemists have no trouble at all constructing a molecular graph as defined above Indeed, use of molecular graphs is one of the most common methodologies in chemistry

C *Topological stereoisomers*

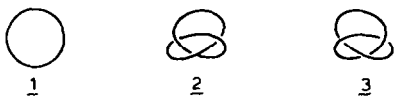
Most isomers fall into one of two categories Constitutional isomers differ in bond connectivity They are distinct by virtue of the topology of the molecular graph For example, as shown in Scheme 2(a), the molecular graphs of n-butane and isobutane are not homeomorphic and not isotopic The famous English mathematician Cayley made a large impact on the chemical world in the 1850s when he showed that the number of possible constitutional isomers with formula $C_nH_{2n+2}$ could be determined utilizing topological arguments This number is, of course, independent of any metric properties of the molecular graphs and is a topological invariant given the rules of valency of carbon

Most stereoisomers, however, are distinct by virtue of some kind of molecular rigidity Euclidean geometrical properties of the molecular graphs are responsible for the distinct character of, e g enantiomers possessing a tetrahedral stereocenter,[7] or *E,Z*-isomers of alkenes, as shown in Scheme 2(b) While very many novel examples of stereoisomerism have been observed, they almost invariably are simply manifestations of different types of molecular rigidity In all such cases, the molecular graphs of a pair of stereoisomers are isotopic and homeomorphic

There is, however, a class of isomers fundamentally different from either of these two classes By definition, all stereoisomers have homeomorphic molecular graphs But, as first discussed in the literature by Wasserman in 1961,[2a b] there are stereoisomers requiring no molecular rigidity to remain distinct *Such stereoisomers have topologically distinct molecular graphs when embedded in 3-space—they are homeomorphic, but not isotopic* As shown in Scheme 3, the prototypal example of such isomerism involves molecular knotted rings If the constructions shown in Scheme 3 represented isomeric molecules, e g cycloalkanes, then clearly they would be stereoisomers They have identical bond connectivity, yet they may not be made congruent Indeed, these stereoisomers would differ from all conventional isomers (stereoisomers and constitutional isomers) since *no* continuous deformation




Scheme 2 Conventional isomers



Scheme 3 Topological stereoisomers

of the molecular graph in 3-space will ever allow them to interconvert (not isotopic), yet they have identical bond connectivity (homeomorphic)

We term such isomers *topological stereoisomers* Furthermore, we define *topological enantiomers* as topological stereoisomers which may achieve mirror image presentations such as the trefoil knots **2** and **3** Topological enantiomers are, of course, *topologically chiral* A construction is topologically chiral if any presentation is topologically distinct from its mirror image [8] *Topological diastereomers*, on the other hand, are topological stereoisomers which cannot achieve mirror image presentations, such as either of the trefoils **2** or **3** and unknotted ring **1** Pairwise comparisons of constitutional isomers, conventional (Euclidean) stereoisomers, and topological stereoisomers in the context of the topology of the molecular graphs are summarized below

Constitutional isomers not homeomorphic, not isotopic
Euclidean stereoisomers homeomorphic, isotopic
Topological stereoisomers homeomorphic, not isotopic

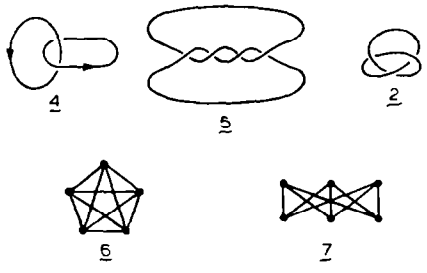
D *Criteria for topological stereoisomerism*

In the following discussion, topological stereochemistry of molecules will be examined in terms of four *elements of topological dissymmetry* It is conjectured that at least one of these elements is necessary for topological chirality (1) an oriented link, (2) any other chiral link, (3) a chiral knotted ring, and (4) a chiral non-planar graph [9]

Scheme 4 illustrates these elements of topological dissymmetry Construction **4**, consisting of two interlocking rings each of which is *oriented*, is the prototypal chiral object in topology One ring serves to "orient the space", and the other defines the configuration of the chiral link Both rings must be oriented for the link to be topologically chiral This construction cannot be transformed into its mirror image by any continuous deformation in 3-space Construction **5** is a link with a minimum of four crossings Again, it consists of two interlocked rings In this case, however, the rings need not be oriented in order for the construction to be topologically chiral Construction **2** is the familiar trefoil knot In topology, any closed curve is a knot It has been demonstrated that very many knots are topologically chiral Many, however, are topologically achiral or amphicheiral in topological parlance A simple circle is an example of an achiral knot Finally, constructions **6** and **7** are non-planar graphs A discussion of these fascinating topological entities is deferred until after a short description of intrinsic and extrinsic topological properties

E *Intrinsic and extrinsic topological properties*

In the foregoing discussion, the phrase "embedded in 3-space" has appeared several times A short explanation of the reason why this embedding is so important to the definitions presented above is interesting and justified at this point even if slightly repetitious Intrinsically, i e ignoring embedding in any space, the knotted ring is topologically equivalent to an unknotted circle, or to its own mirror image or, indeed, any other knot![10] In a space with dimensionality higher than three, these constructions may



Scheme 4 Illustration of some elements of topological dissymmetry

be interconverted by continuous deformation The knotted ring could be mathematically taken into 4-space, deformed *without breaking the line*, then returned to 3-space as an unknotted ring Intrinsically equivalent constructions are homeomorphic All knots are homeomorphic to a circle Likewise, a link is indistinguishable from two unlinked rings *intrinsically All* stereoisomeric molecular graphs must be intrinsically topologically equivalent or homeomorphic, while all constitutional isomers have intrinsically distinct molecular graphs

But, embedding in 3-space imparts *extrinsic topological properties* to a construction Two constructions which are topologically equivalent in 3-space are termed *isotopic* All isotopic constructions are homeomorphic Not all homeomorphic constructions, however, are isotopic The distinct character of the mirror image trefoil knots, or of the link and two separated rings, are *extrinsic topological properties* These constructions are homeomorphic, but not isotopic Any topological chirality must necessarily be a property of embedding of an object in some space, and is an extrinsic property—all mirror image objects are homeomorphic Topological stereochemistry involves a collection of extrinsic topological properties deriving from the embedding of molecular graphs in 3-space Again, topological stereoisomers have *homeomorphic, but not isotopic* molecular graphs

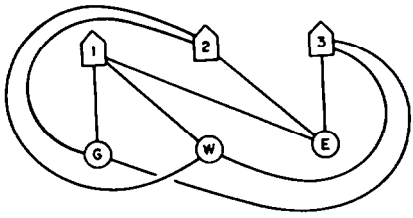
F *Non-planar graphs*

As mentioned above, a graph is simply any construction composed of points and lines A subgraph may be derived from a graph by selective removal of points and lines Constructions **2**, **4** and **5** can be considered graphs with no points although such closed curves usually are not considered graphs Of course, some points may be embedded in the closed curves If the latter is the case, then the resulting graphs are termed *circuits* If three differently colored points are available, then a graph equivalent to construction **4** can be generated in 3-space by embedding the three points in each of the two circles In the following discussion, the terms "link" and "knot" are used interchangeably with "linked circuit graph" or "knotted circuit graph" This is not precise, but since all molecular structures are graphs, we need not consider closed curves outside the context of circuit graphs

Graphs can be divided into two categories Some graphs have possible presentations which may be projected in a plane without any crossings, e g the molecular graphs of the butanes shown in Scheme 2(a) These are termed *planar graphs* A little experimentation with pencil and paper will serve to demonstrate that many more complex graphs, such as that for cubane, are also topologically planar On the other hand, some graphs *have no presentation* allowing projection in a plane without at least one crossing A crossing here is defined as any point in the plane projection of the graph where two edges cross, but there is not a vertex In order to differentiate such crossings from actual points (vertices) which are part of the graph, the points of the graph are usually embolded Alternatively, for graphs embedded in 3-space such crossings can be represented by a "break" in one of the lines the one passing "underneath" The latter convention is, of course, very common in chemistry The convention is used to obvious benefit in the drawings of structures **2**, **4**, and **5**

The graphs corresponding to links and knots are non-planar because of the embedding in 3-space However, they are homeomorphic to circuits which may be projected onto a plane with no crossings Intrinsically, constructions **2**, **4**, and **5** are planar! This contraintuitive statement is simply a mathematical result of the fact that the constructions are homeomorphic to planar presentations There are, however, graphs which are intrinsically non-planar The famous Polish topologist Kazimierz Kuratowski first showed that *all* such non-planar graphs contain as a subgraph one of only two basic non-planar graphs—the first and second Kuratowski graphs **6** and **7**, respectively [5] Harary, of the Department of Mathematics at the University of Michigan, has named these graphs $K_5$ (**6**), and $K_{3,3}$ (**7**) [11] The "standard" presentations of $K_5$ and $K_{3,3}$ are shown in Scheme 4 The first Kuratowski graph $K_5$ is simply the complete graph on five vertices That is, each point is joined to every other by an edge The second Kuratowski graph $K_{3,3}$ is the complete bi-partite graph on two sets of three vertices each

The latter graph has also been called the graph of three houses (one set of three vertices) and three utilities (the other set of three vertices) [5a] This model provides an interesting illustration of the topological non-planarity of these constructions Thus, as shown in Scheme 5, consider a housing project with three houses 1, 2, and 3 Each house must be connected to the utilities gas, water and electricity Suppose, as the story goes, that the prospective owners are feuding and require that their utilities be connected without any of the lines crossing The unhappy construction engineer sits down with a pencil and paper and tries to design such a layout The engineer is thwarted, however, since it is




Scheme 5 $K_{3\,3}$—the graph of three houses and three utilities

topologically impossible to connect three houses to three utilities without *at least one crossing* One solution with only one crossing is shown in Scheme 5 It should be noted that this is not an extrinsic property, but is actually independent of any embedding space Intrinsically the "normalization" of the crossings, that is, which line is above and which is below, has no meaning But the graph still must possess at least one crossing in any plane projection

To allow easy recognition of the $K_{3,3}$ graph, it is useful to note that the vertices of $K_{3\,3}$ can be colored such that each vertex of one color is joined to all three of the other, but not to any of its own color Thus, each house is joined to each utility and each utility is joined to each house No house, however, is directly joined to another house and no utility is directly joined to another utility This graph is termed "minimally non-planar", since it may achieve a presentation with only one crossing A little experimentation will also serve to convince the reader that the $K_5$ arrangement is also minimally non-planar Any non-planar graph, no matter how many crossings it must have, may be reduced to one of these two graphs by selectively removing edges

Many of the properties of the Kuratowski graphs are known But, graph theorists have done very little exploration of the extrinsic topological properties of graphs deriving from an embedding in 3-space Since topological chirality is just such a property, the topological chirality of graphs is not a well-studied area of graph theory or low-dimensional topology As described below, it is conjectured that some molecular graphs may be topologically chiral *without* possessing any of the more conventional elements of topological dissymmetry 1, 2, or 3 It is further conjectured that such molecular graphs must possess either $K_{3,3}$ or $K_5$ as a subgraph [9] Thus, the non-planar graphs are included in Scheme 4 as elements of topological dissymmetry of a fourth kind

G *Criteria for topological chirality and diastereoisomerism—revisited*

The necessary and sufficient condition for topological chirality is that any presentation of the construction be topologically distinct from its mirror image This implies that no presentation may be converted into its mirror by continuous deformation in 3-space Mathematically, topological chirality may be proved by methods which are far outside the scope of this report Some simple generalizations, however, are very useful for proving the topological achirality of molecular graphs

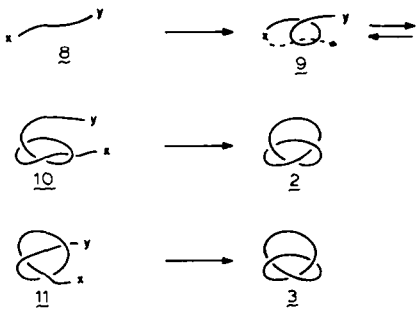
If any *rigidly achiral presentation* is found for a construction, topological chirality is ruled out Of course, such a presentation must possess an improper axis of symmetry [12] This constraint readily allows topological chirality to be ruled out for most molecular graphs Specifically, any *planar* presentation of a molecular graph is rigidly achiral in 3-space It possesses at least one $\sigma$ plane (the plane in which it is embedded), and is therefore a rigidly achiral presentation Most topologically non-planar one-dimensional objects contain either a link, knot, or non-planar graph [9] The non-planar graphs are intrinsically non-planar, the links and knots are non-planar when embedded in 3-space This is the reason for choice of the links, knots, and non-planar graphs as elements of topological dissymmetry The separation of the oriented link from other chiral links is arbitrary, but useful in the following discussion

Concerning criteria for topological diastereoisomerism, it is more difficult to generalize It is conjectured, however, that for two constructions to be topological diastereomers, *at least one must be non-planar*, though neither need be topologically chiral Another way of stating this conjecture is the following two planar homeomorphic constructions must be isotopic in 3-space

## PART II CHEMICAL SYNTHESIS OF TOPOLOGICAL STEREOISOMERS BY THREADING

A *Introduction*

Somewhat surprisingly, topological stereoisomerism is extremely rare outside the DNAs Indeed, of all the pure non-polymeric compounds ever synthesized or isolated (ignoring DNAs) we know of



Scheme 6 The trefoil knot by threading

only three examples of topologically chiral molecules, and one example of topological diastereoisomerism! This section describes the synthesis of these topological stereoisomers, plus other chemistry closely related to topological stereochemistry While there are almost certainly other examples of topological stereoisomerism in the literature of which the author is not aware, the following discussion will serve to illustrate the novelty of these structures

B *General considerations*

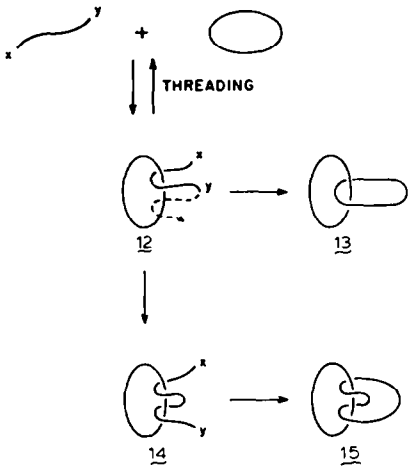
Consider approaches to the synthesis of a knotted ring As shown in Scheme 6, one obvious strategy involves simply cyclization of a linear molecule functionalized such that the ends may become bonded Thus, a linear molecule (**8**) may easily achieve the presentation **9**, called a *loop* For a real molecule, this conformation does not seem unlikely if there are many atoms in the chain The next operation may be termed a *threading* Passing one end of the chain through the loop gives the construction **10**, called a *twist* [3] This sort of construction in lay language could be called a knot (the sailors half-hitch) But in topology a knot *must* be a closed curve Notice that **8**–**10** are all isotopic Finally, formation of a covalent bond between X and Y gives the trefoil knot **2** A series of mirror image events would afford the alternative trefoil, as shown for **11** → **3** The hypothetical enantiomeric transition states leading to **2** and **3** may be correctly said to have different topology

In real chemical systems, knotted rings must certainly have been synthesized many times since efficient methods for macrocyclization reactions were first developed The amount of knotted rings, however, must be very small Part of the problem is in the threading process itself In real chemical systems, such a threading process is unlikely because of the conformational properties of the chains A "twist" conformation in which the loop is threaded must be disfavored relative to the unthreaded conformation, at least in saturated hydrocarbon systems In addition, problems in isolation and characterization of the knotted product are difficult to overcome To our knowledge, outside of DNA chemistry no knotted ring has ever been isolated or characterized

C *Catenanes by threading*

A threading approach to synthesis of molecular linked rings, termed *catenanes*, similar to that described for the trefoil knot, is also evident as shown in Scheme 7 Thus, threading of a chain through a pre-formed ring can give construction **12** If the ends of the chain are now functionalized such that they cannot slip back through the ring, **12** becomes a *hooplane* or *rotaxane* [2c 13] Note that no matter what X and Y are, construction **12** is isotopic with the separated chain and ring If the ends of the chain in **12** become bonded, then a link (**13**) results The link is topologically distinct from the unlinked ring components when embedded in 3-space A second threading of **12** could give the "double-looped" construction **14**, which in turn could cyclize to give the *double-looped catenane* **15** For the case of formation of rotaxanes **12** and catenanes **13** by threading as shown in Scheme 7, useful mathematical modeling of the probability of threading and catenane formation has been accomplished [2a 14–17]

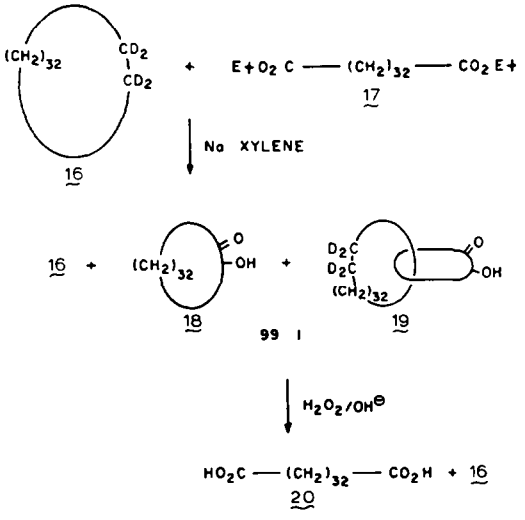
Chemically, achieving a synthesis of a catenane by the threading approach outlined in Scheme 7 is a two-fold problem (1) macrocyclization to give cyclic molecules with greater than 20 atoms and (2) isolation of the linked product According to Prelog, Willstatter discussed the possibility of synthesis of linked macrocyclic rings in a seminar at Zurich sometime between 1900 and 1912 [18] Good solutions to the problem of macrocyclization were not available until the 1950s, however In the middle and late 1950s apparently at least five groups were simultaneously attacking the problem of synthesis of linked



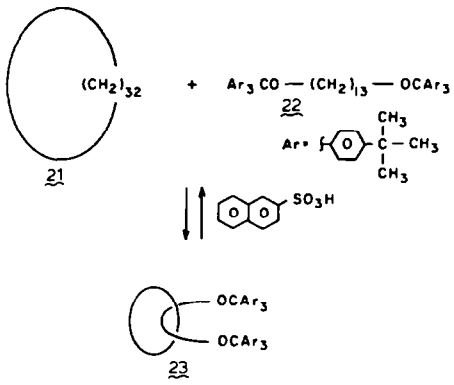

Scheme 7 Catenanes by threading.

molecular rings They were the group of Luttringhaus (a Ziegler student who had been involved in the early work on synthesis of macrocycles) at Freiburg University, Cramer at Heidelberg, Kohler and Dietrich at Tubingen, Wasserman at Bell Laboratories in Murray Hill, and Van Gulick at the University of Oregon [2c,5,21] The first publication concerning an (unsuccessful) attempt at catenane synthesis was by the Luttringhaus group in 1958 [19] Though no catenane product was isolated in pure form at the time, Wasserman first convincingly demonstrated synthesis of a catenane and reported his results to the Division of Polymer Chemistry at the 138th Meeting of the ACS in New York City in September of 1960 A communication describing that work appeared in August of that year [20] He and van Gulick independently coined the term catenane (Latin, *catena*, chain) in the late 1950s [21]

1 *The Wasserman synthesis* Because of the historical importance of this synthesis in the field of topological stereochemistry, a short discussion of the Wasserman catenane synthesis is presented Since macrocyclization methodology by 1960 was quite well established, the problem had become one of convincing demonstration of the existence of the linked products This was accomplished in a very clever way As shown in Scheme 8, acyloin condensation of diethyltetratriacontanedioate (**17**) in the presence of an equimolar amount of the deuterated cyclotetratriacontane (**16**—the material utilized contained an average of 5 D atoms/molecule) in xylene solution gave greater than 70% of acyloin **18** Chromatography allowed facile separation of the macrocycle **16** from the acyloin **18** However, the twice chromatographed acyloin product still contained carbon–deuterium bonds by IR spectroscopy




Scheme 8 The Wasserman catenane synthesis

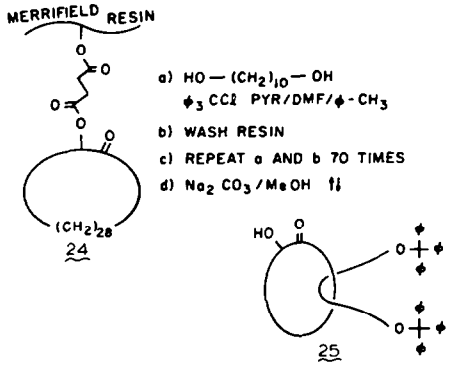



Scheme 9 The first Harrison rotaxane synthesis

Oxidative cleavage of the acyloin product gave the diacid **20** *plus approximately* 1% *of the cycle* **16** The identity of the material obtained after this oxidation with the starting macrocycle **16** was shown by m p and mixed m p These results are best interpreted by assuming the existence of catenane **19** in admixture with the acyloin product **18** produced in this reaction Indeed, isolation of pure catenane prepared by this approach was later reported in the literature,[2b] thus demonstrating threading and formation of linked product by the approach shown in Scheme 7 for the first time The actual amount of linked product produced in this way was shown to be in reasonable agreement with that predicted based upon a random coil model [2a] Wang, working with DNAs, was able to show that for DNA strands, this mathematical model gave excellent agreement with experiment [14]

2 *More rotaxanes and catenanes by threading* More work on understanding the threading process has been accomplished in the years since 1960 Harrison carried out an interesting series of experiments designed to determine the structural parameters affecting the threading equilibrium in hydrocarbon rings and chains [16,22] As would be expected, under equilibrating conditions the amount of threaded product increases with increasing ring size Thus, as shown in Scheme 9, when a neat mixture of cyclodotriacontane (**21**) and 1,13-di(tris-4-t-butylphenylmethoxy)tridecane (**22**) is heated with naphthalene-$\beta$-sulfonic acid, equilibrium is established between the rotaxane **23**, the free cycle and chain After trapping the equilibrium mixture with triethylamine, then purification of the products by chromatography on silica gel, 1 1% yield of the crystalline rotaxane (**23**) is isolated With cyclotritriacontane ($C_{33}H_{66}$) a 1 6% yield of rotaxane is obtained under similar conditions The method could not be utilized above the cyclo ($C_{33}$) material since for higher rings the threaded product was thermally unstable With the larger ring sizes the triaryl end groups can slip through the ring rapidly on the isolation time scale at room temperature

A novel and interesting application of polymers in organic synthesis resulted in a rotaxane synthesis by Ian and Shuyen Harrison [13] As shown in Scheme 10, treatment of covalently polymer bound cyclotriacontane acyloin (**24**) with 1,10-decanediol, triphenymethyl chloride and pyridine, then washing of the resin, gave a very small amount of polymer-bound rotaxane Repetition of the cycle 70




Scheme 10 The Harrisons' polymer-bound rotaxane synthesis

times, then cleavage of the macrocycle from the resin afforded a mixture from which 6% of pure rotaxane **25** was isolated as an oil

All of the above results serve to confirm the basic correctness of the calculations indicating that with hydrocarbon chains, the threading approach is a very inefficient method for preparation of catenanes or knots It is expected, however, that changing the chemical nature of the chain and ring may change the likelihood of threading, and make catenane synthesis by the threading approach more efficient

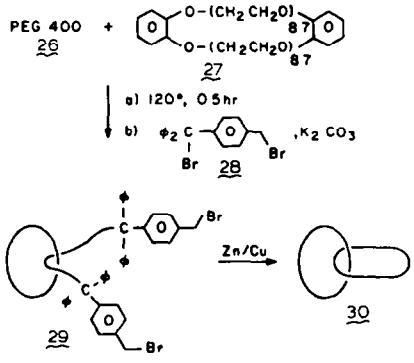
Indeed, some very interesting work on threading in macrocyclic *polyethers* was reported in 1976 by Albert Zilkha and his co-workers at the Hebrew University of Jerusalem This work has culminated in an efficient total synthesis of catenated rings by the threading approach,[17 23] though unfortunately, commercial mixtures of polyethyleneglycols (PEGs) and non-homogeneous crown ethers derived therefrom were used in all of the experiments

Treatment of a mixture of crown ether rings and PEG with naphthalene-1,5-diisocyanate gave high molecular weight polyurethane polyrotaxanes Any ring not threaded could be washed out of the polymer and quantitatively determined, affording a method for trapping of the threaded materials and measurement of the extent of threading Using this technique, optimum conditions for threading at equilibrium were found Thus, $76 \pm 2\%$ of the rings introduced are threaded at equilibrium with dibenzo-58 2-crown-19 4 and PEG 600 (average 13 2 ethylene glycol units per chain, molar ratio ring/chain of 0 5)[124] Also, it was shown that the threading equilibrium is reached after 0 5 hr at 120°

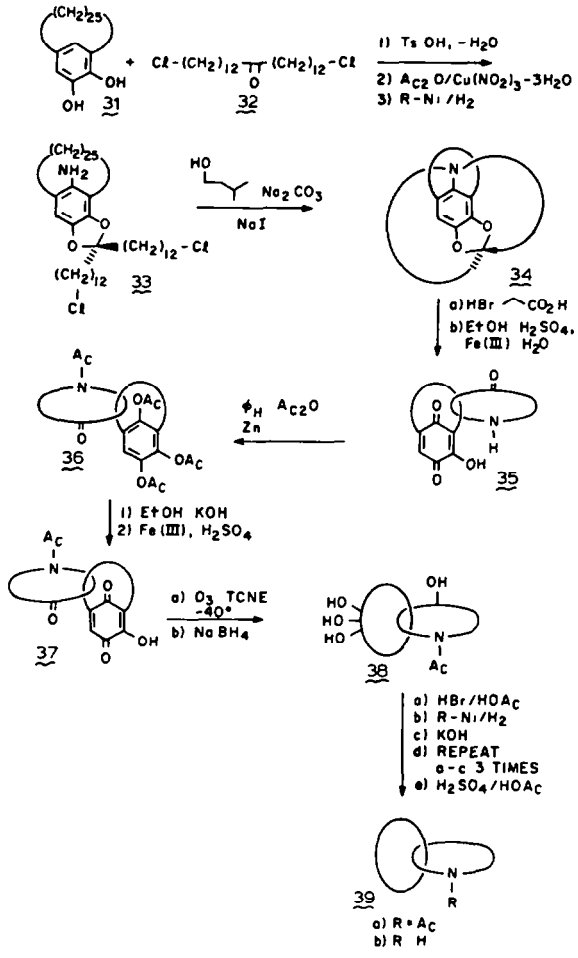
This work led to the catenane synthesis shown in Scheme 11 [23] Treatment of PEG 400 (**26**) and the mixture of crown ethers **27** under conditions first to establish threading equilibrium, then to cap the ends with a functionalized triarylmethane **28**, gave the rotaxane **29** in 18 5% isolated yield Benzylic coupling promoted by zinc/copper couple in DMF then gave the catenane **30** in 14% yield The synthesis proceeds in two steps and 2 6% overall yield from readily available starting materials

3 *The intramolecular threading approach the Schill–Luttringhaus catenane syntheses* The first directed synthesis and detailed characterization of a catenane was accomplished by Schill and Luttringhaus in 1964 [2c 25] The work of Luttringhaus and his students up to about 1970 has been reviewed in great detail by Schill [2c] Several catenanes have been prepared utilizing the clever Schill–Luttringhaus approach, which is exemplified by the syntheses shown in Scheme 12 [25 26] Treatment of the readily available bicyclic catechol **31** with the ketone **32** under standard ketalization conditions, followed by nitration and reduction gives the amino dichloride **33** Intramolecular macrocyclization of this material gives the 3° amine **34** containing a linked circuits subgraph Note that construction **34** is non-planar in 3-space In this synthesis, the Euclidean geometry of **33** is utilized to achieve topological stereocontrol, since alkylation of the amino group from the same face of the macrocyclic ring with both alkyl chloride chains is apparently disfavored sterically, favoring the "threaded" product over the unthreaded topological diastereomer Indeed, no diastereomeric compound has been isolated utilizing this strategy Interestingly, if the alkyl chloride chains are increased in length, the macrocyclization fails to occur, and if the polymethylene macrocycle is too short (19 methylene units), the aryl–nitrogen bond could not be cleaved [26d]

Hydrolysis of the ketal moiety, then oxidation of the aromatic nucleus with iron(III) gives the catenane **35** Because of the instability of the quinone product **35**, the catenanes prepared in this manner are generally isolated as rearomatized acetates exemplified by **36** The rearomatization is readily




Scheme 11 Polyether rotaxanes and catenanes by threading.

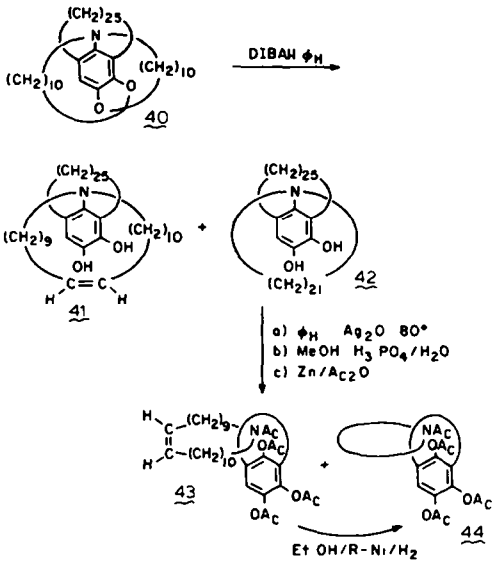


Scheme 12 The Schill–Luttringhaus catenane synthesis

accomplished by treatment of the crude hydroxyquinone with zinc and acetic anhydride For catenane **36**, one of the circuits in the link is directed but the other is not The link is therefore topologically achiral Schill has successfully converted **36** to the more pristine catenanes **39** as shown in Scheme 12 Thus, selective hydrolysis of the acetoxy groupings in the presence of the amide function, followed by oxidation of the aromatic ring gives the hydroxyquinone amide **37** Ozonolysis, then borohydride reduction of the crude tetraketone ozonolysis product affords the tetrol **38** A halogenation–hydrogenolysis procedure serves to remove the hydroxyl functionality, affording the crystalline catenane **39a** Hydrolysis of the amide grouping finally gives crystalline **39b** Carbon longitudinal relaxation time ($T_1$) measurements on catenane **39a** indicate that, as expected, the motions of the hydrocarbon ring in the catenane are restricted relative to those in a free macrocycle, but not completely correlated as in a rigid molecule [27]

Interestingly, an alternative method for cleavage of the precatenane has afforded some novel topological stereoisomers, though only as components of a mixture As shown in Scheme 13, treatment of the precatenane **40**, prepared as described for **34**, with DIBAH gives the mixture containing amines **41** and **42** Cleavage of the aryl–nitrogen bond by the standard Schill methodology, or by the modification indicated in the scheme, gives the catenanes **43** and **44** Note that in pre-catenane **41** and catenane **43**, both rings of the link are directed! Thus, compounds **41** and **43** actually exist as racemic mixtures of topological enantiomers The topological chirality in this case derives from topological dissymmetry of type 1 To our knowledge this type of topological isomerism in small molecule chemistry is unique to these particular compounds Hydrogenation of the mixture of **43** and **44** gives the topologically achiral link **44** in pure form [28]

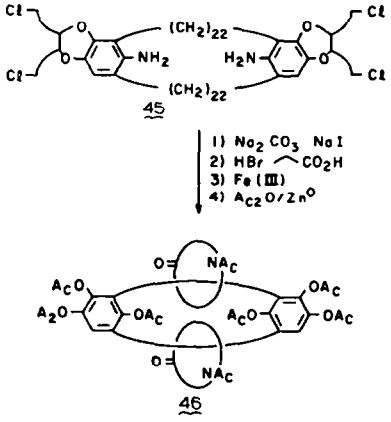
The Schill approach has been extended in a straightforward way to the [3]-catenane **46**, as shown in Scheme 14 Cyclization of the diamino hexachloride **45**, followed by the standard Schill cleavage



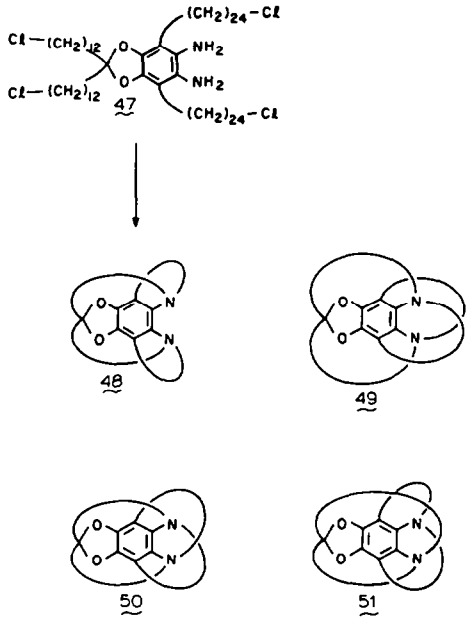
Scheme 13 Topological stereoisomerism in the Schill catenanes

procedures gives the 3-catenane **46** in moderate yield Interestingly, the three-ring link **46** is produced along with an inseparable mixture of two "translation isomers" where both macrocyclic ketoamide rings are on the same side of the aromatic rings These compounds are not interconverted, nor converted to **46**, at 80° [29]

Attempts by Schill to extend the strategy to synthesis of a knotted ring have so far been unsuccessful,[30] though some interesting results have been obtained Thus, as shown in Scheme 15, cyclization of the novel tetrachloride **47** is expected to give four products, **48–51** In fact, three crystalline materials are isolated in low yield (A, 0 8%, B, 0 52%, and C, 0 37% yields of sharp melting materials after chromatography and recrystallization) [31] The symmetry properties of the molecules allow assignment of structure **51** to compound C This is the only one of the three products with no $C_2$ axis as evidenced by the $^1$H- and $^{13}$C-NMR spectra Unfortunately, firm structural assignment for the other two products has not proven possible The spectra of compounds A and B are most consistent with **48** and either **49** or **50** It is not clear why four products are not isolated Note that **49** and **50** are topological diastereoisomers, and **50** is non-planar and topologically chiral because of a knotted circuit subgraph (topological element of dissymmetry of type 3) Cleavage of the Ar—N bonds and ketal hydrolysis of **50** would yield a trefoil knot Further work, including X-ray analysis of these products, is under way in the Freiburg laboratories



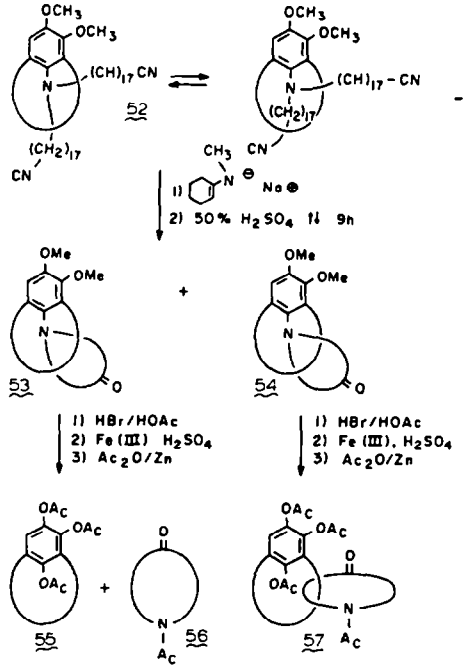
Scheme 14 Synthesis of a [3]-catenane



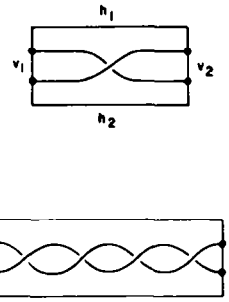
Scheme 15 Towards a knot by the Schill approach

D *Topological diastereomers in the catenane literature*

In order to increase the probability of catenane formation relative to the intermolecular threading approach, the Luttringhaus group tried several approaches to macrocyclization of a chain already covalently attached to a macrocyclic ring This work finally led to the Schill syntheses as described above There was, however, another intramolecular threading approach that led to isolation of catenated products, but did not give the complete stereocontrol of the Schill approach Interestingly, because of its lack of stereocontrol, this synthesis, published by Luttringhaus and Isele in 1967, led to the *first isolation of topological diastereomers ever reported* [32] As shown in Scheme 16, treatment of the presumably equilibrating mixture of t-amines **52** under Ziegler's conditions for macrocyclization of



Scheme 16 The first topological diastereomers




Scheme 17 Two-braids with one and four crossings

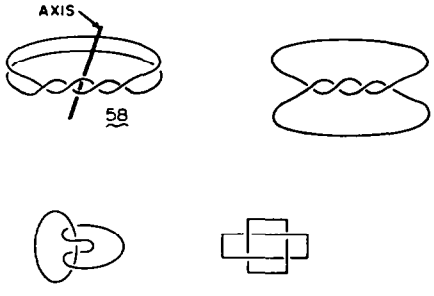
dinitriles gave two products, the extraannular bicyclic **53**, and the intraannular link **54** *These compounds are topological diastereomers* Notice that one of them is non-planar because of a linked circuits subgraph Recall the conjecture that for topological diastereomers, at least one must be non-planar There is, however, no topological chirality Interestingly, the presumed intermediate in formation of **54**, the α-cyanoimine, possess two oriented circuits, and would therefore exist as a racemic mixture of topological enantiomers Cleavage of the Ar—N bonds of **53** and **54** utilizing Schill's chemistry gave the separated rings **55** and **56**, and the catenane **57**

## PART III THE MÖBIUS STRIP APPROACH TO SYNTHESIS OF TOPOLOGICAL STEREOISOMERS

### A *Braids and wreaths*

In the next section, a discussion of the concept and realization of what has become called the "Möbius strip approach" to the synthesis of the topological stereoisomers will be presented In order to allow better appreciation of this, a short discussion of the topology of *braids* and *wreaths* is presented [3] Braids are special presentations of constructions composed of threads and a four-sided polygon Consider a rectangular "frame" with two vertical sides $v_1$ and $v_2$, and horizontal sides $h_1$ and $h_2$, as shown in Scheme 17 A braid is formed when threads are stretched from $v_1$ to $v_2$ in a special way Thus, each thread must connect with each vertical side once, defining points For a braid with two threads (two-braid), each vertical side will have two points The threads may cross each other, but no thread may cross a horizontal or vertical side in a planar projection Finally, a line from $h_1$ to $h_2$ crosses each thread exactly once (avoiding the crossings of the braid itself) in a planar projection, loops are not allowed Tow-braids with one and four crossings are shown in Scheme 17

Wreaths are formed when the rectangular frame is bent around to form a cylinder, such that $v_1$, $v_2$, and the corresponding points become congruent The threads are allowed to leave the surface of the cylinder, but cannot cross its axis Construction **58** in Scheme 18 depicts one possible presentation of the *two-braid wreath* with four crossings The cylindrical frame is not shown, but the "impassable" axis is shown In general, if the *crossing number* is greater than zero, we conjecture the wreath with its impassable axis is topologically chiral If the number of crossings in the two-braid is even or zero, the corresponding wreath is composed of two closed curves These curves are linked if the crossing number is non-zero If the crossing number is odd, then a single closed curve results upon wreath formation If the crossing number is greater than 1, then the curve is a non-trivial knot [33]




Scheme 18 Two-braid wreath with four crossings

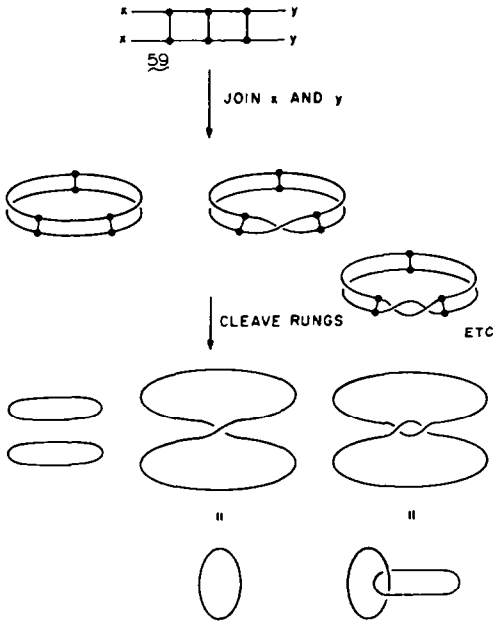
If one now removes the axis of the cylinder of a wreath deriving from a two-braid, the resulting construction is a member of the class of "two-braid knots and links" Several well-known presentations of the two-braid link with crossing number four are shown in Scheme 18 Note that the minimum crossing number is a topological invariant of this construction The whole class of two-braid knots and links are very familiar topological entities Further discussion of the individual members of this class is given below

B *The Mobius strip approach*

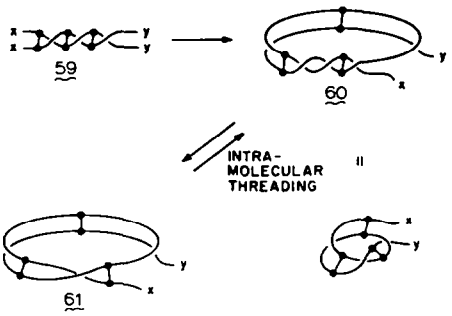
In the late 1950s Wasserman at Bell Labs, and van Gulick at the University of Oregon, independently conceived of and wrote on an approach to the synthesis of knotted rings, chiral catenanes, and other topologically interesting structures which is arguably more elegant than any of the approaches described above [2,21] From the point of view of directed total synthesis, one may consider this "Mobius strip approach" to be a novel example of *stereocontrol* As shown in Scheme 19, in its simplest form the idea involves performing a bis-macrocyclization reaction on a ladder-shaped molecule composed of two long chains of atoms (the edges of the ladder) joined by several connecting moieties (the rungs), as shown schematically for the three rung case in structure **59** The chains are functionalized such that they may be bonded end to end, and the Euclidean geometry of the structure is such that an end may not pass between two rungs

After intramolecular reaction, wreath-like products of cyclization would result Depending upon the length of the chains and other Euclidean considerations, the resulting products could be twisted Three products, with zero, one, and two half-twists, are shown Purification of these twisted products, then cleavage of the rungs would afford a subset of the two-braid knots and links as shown, showing a plethora of novel topological stereoisomerism Indeed, the products before cleavage of the rungs represent some of the most novel examples of topological stereoisomerism (the Mobius ladders and chiral prisms), as discussed below

For obvious reasons, this synthetic approach was termed the Möbius strip approach Though the degree of twisting of the molecular ladder in this synthesis relies to some extent on randomness in the initial and second cyclization events, it is clear that a vastly more efficient preparation of knots and links may be achieved in this manner relative to starting with single stranded precursors no intermolecular or intramolecular threading is required, but rather a *much more readily achieved twisting* As shown in Scheme 20, the intermediate in formation of a knot would be the singly-closed construction **60** Note that this construction has a *twist* subgraph, but that no threading of a chain through a loop was required for its formation Similarly, the corresponding intermediate with two crossings, leading to a link, has a




Scheme 19 The Möbius strip approach

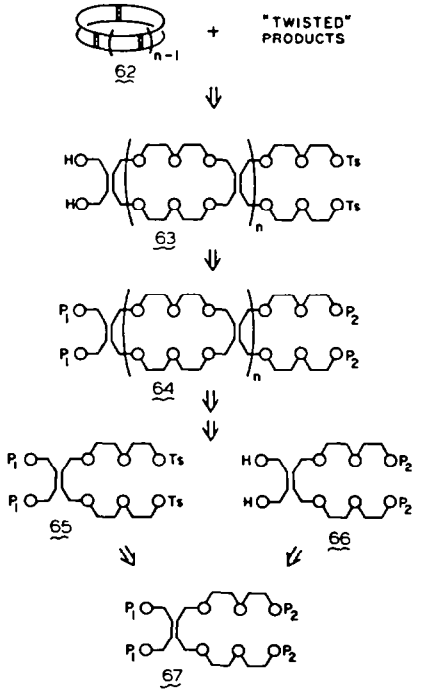



Scheme 20 Formation of a trefoil knot by the Möbius strip approach

ring with a chain threaded through it, though no "threading" took place As indicated in Scheme 20, if the second macrocyclization step is slow relative to the rate of equilibration between conformers **60** and **61**, then the Mobius strip approach is a special example of intramolecular threading The esthetically pleasing nature of the Möbius strip approach has captured the imagination of many chemists in the 20 years since it was first described in the literature Until recently, however, no group had achieved a synthesis along these lines in the laboratory

1 *The THYME polyethers* A strategy for chemical realization of the Mobius strip approach has recently been developed in our laboratories, as shown in Scheme 21 [34] Any such strategy must confront several problems First, a decision regarding the chemical composition of the edges of the ladder, and more importantly, the rungs, must be made Then, chemistry for generating the functionality required for the macrocyclization process must be developed

For reasons having to do with another rationale for this work (host–guest chemistry[35]), it was first decided that the edges of the ladder would be composed of polyethyleneoxy chains For the crucially important rungs, a tetraether of tetrahydroxymethylethylene (THYME) was chosen Thus, it was envisioned that molecular "wreaths" **62** would derive from bis-macrocyclization of diol-ditosylates of type **63** Compounds **63** would, in turn, derive from selective deprotection of a protected tetrol precursor **64** The rest of the strategy, as shown, derives in a straightforward way from consideration of convergence [36] Synthesis of **64** ($n = 1$) derives from intermolecular coupling of two fragments **65** and




Scheme 21 The THYME polyethers

**66** The latter, in turn, derive from selective deprotection and coupling of acyclic tetrol **67** Preparation of compounds of type **64** where $n > 1$ may be readily envisioned by application of the strategy where the ditosylate and diol derived from selective deprotection of **64** ($n = 1$) replace **65** and **66** The protecting groups $P_1$ and $P_2$ must be orthogonal,[37] removable in the presence of the THYME tetraether functionality, and $P_1$ must be removable in the presence of a 1° toluenesulfonate grouping

This strategy has many advantages Firstly, it is in principle highly efficient, and readily amenable to preparation of products with varying lengths of the chains comprising the edges of the ladder Also, the Williamson etherification reaction utilized for the crucial macrocyclizations is known to be one of the most efficient macrocycle forming processes available Recall the efficient preparation of catenanes based upon use of PEGs Finally, the choice of ethyleneoxy chains for the edges becomes especially beneficial since the oxygen functionality is expected to allow separation of the twisted isomers The latter point is very important since separation of a mixture of hydrocarbons of type **62** is expected to be problematical

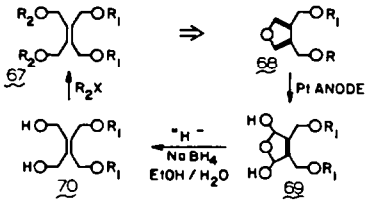
The THYME unit too possesses many distinct advantages for use as a linking group It has no tetrahedral stereocenters,[7] and thereby side-steps complications arising from diastereomer formation in the coupling process **65** + **66** → **64** Also, the THYME unit has considerable molecular rigidity, presumably aiding in the macrocyclization steps Finally, the double bond is easily cleaved—a necessary feature of the rungs for implementation of the Möbius strip approach to synthesis of the two-braid knots and links

As shown in Scheme 22, an efficient method for preparation of THYME tetraethers of type **67** has been developed [34] The synthesis depends upon the excellent procedure for anodic oxidation of furans developed by Magnusson [38] Anodic oxidation of a furan of type **68** affords directly the bis-hemiacetal **69** Mild borohydride reduction then gives THYME diol **70**, that is easily converted in a regioselective manner to the tetraether **67** by Williamson etherification This scheme for generating the THYME diol function **70** from furan **68** is quite mild chemically, and may be accomplished in the presence of a tetrahydropyranyloxy (THP) protected alcohol, and in the presence of the toluenesulfonate (TsO) functionality Thus, the strategy outlined in Scheme 21 may be realized utilizing the furan ring itself as protecting group $P_1$ and the THP grouping as $P_2$

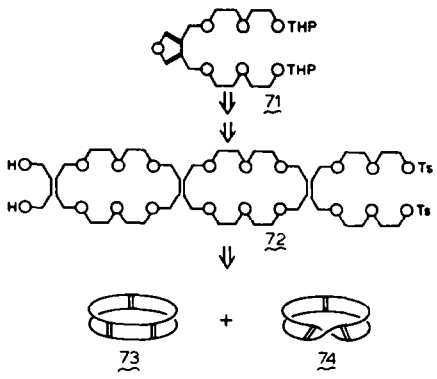
2 *Total synthesis of the first molecular Möbius strip* Utilizing this approach, as indicated in Scheme 23, an efficient synthesis of the tris-THYME diol-ditosylate **72** has been achieved from the key "two-arm" furan-diTHP ether **71** Examination of CPK space-filling molecular models leads to the prediction that three products will be produced upon base-promoted intramolecular cyclization of this material Specifically, equal amounts of the untwisted tris-THYME cylinder **73** and racemic molecular Mobius strip **74** are expected Formation of more highly twisted products is disfavored sterically Gratifyingly, within experimental error, exactly this result is obtained In addition, the cylinder **73** and racemic Mobius molecule **74** are readily separated by flash chromatography on alumina The structure of cylinder **73** is unequivocally established by single crystal X-ray analysis, and the structure of the molecular Mobius strip **74** is firmly established by spectroscopic techniques

The molecular dynamics of **73** and **74** are quite interesting, and deserve some comment Firstly, the $^1$H-NMR of the tris-THYME cylinder **73** shows an AB quartet for the allylic methylene Thus, the cylinder cannot be turning "inside out" rapidly on the NMR time scale In this case, a lower limit on the "lifetime" of a conformational state in which the allylic protons are diastereotopic is about 7 ms [39]

Even more interestingly, the $^1$H- and $^{13}$C-NMR spectra of tris-THYME Möbius strip **74** indicate that this molecule *is* undergoing some novel conformational changes rapidly on the NMR time scale To visualize this motion, consider the structure **74** as embedded in a Mobius surface (see below) The




Scheme 22 Preparation of the THYME tetraethers



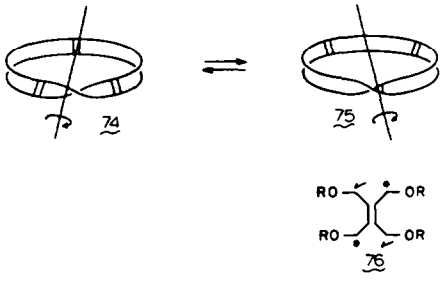

Scheme 23 Synthesis of the first molecular Möbius strip

"twist" of a Mobius band cannot be spread evenly throughout the band, but must be localized in some part of it Consider this "locus of twist" as moving around the band in a sort of pseudo-rotation Now consider what one would see if one were in the rotating reference frame The double bonds would seem to be moving around the surface of the strip much as the ants in the famous Escher print "Möbius Strip II" The THYME double bonds have no "sidedness" in the non-rigid sense, and are rotating end over end relative to the axis of the wreath defined by the edges of the strip

The highest symmetry any single conformation of this molecule may have is $C_2$, as shown in Scheme 24 The conformation shown for structure **74** possesses a $C_2$ axis passing through the center of the "back" double bond This axis of symmetry serves to identify two pairs of allylic carbons, indicated by checks and stars in the drawing **76** If this were the only symmetry the molecule could achieve, then two different allylic carbons would be present in the $^{13}$C-NMR spectrum There is, however, a fundamentally different $C_2$ conformation, as shown in structure **75** In this conformation, one of the double bonds is "in the twist", and the $C_2$ axis passes directly through both olefinic carbons This symmetry serves to identify the checked and starred allylic carbons In fact, only one allylic carbon is observed in the $^{13}$C-NMR spectrum at 62 9 MHz, and only one allylic AB quartet is observed in the $^{1}$H-NMR spectrum at 250 MHz These results are easily interpreted by assuming that conformations with the symmetry of those shown in **74** and **75** are in rapid equilibrium Examination of CPK molecular models indicates that this assumption is very reasonable

The symmetry of **74** may be expressed in terms of a Longuet–Higgins non-rigid molecular symmetry (MS) group [40] This group consists of the set of permutations, permutation-inversions, and rotatory reflections which are feasible under the conditions of measurement For a rigid molecule the MS group is isomorphic to the molecular point group Two or more nuclei are symmetry equivalent if they are permutable under an operation of the MS group [41] Molecular Möbius strip **74**, given the above described conformational flexibility, belongs to the MS group $C_2 \wedge C_6$ This is a group of order 12, isomorphic to $D_6$ In this case the $D_6$ isomorphism has a nice intuitive interpretation, since in a "time averaged" sense, there are six $C_2$ axes perpendicular to the axis of the wreath defined by the edge of structure **74**, and a $C_6$ axis congruent with the axis of the wreath, but no $\sigma$ planes [42] To our knowledge, this non-rigid symmetry is unique to **74**

Of course, **74** is chiral We, as chemists, know this to be the case since examination of essentially all of




Scheme 24 Symmetry of the molecular Möbius strip


Scheme 25 The Möbius ladders $M_6$ and $M_8$

the possible conformations accessible to the molecule under our standard conditions shows that (1) every conformation is chiral, and (2) given the Euclidean constraints on the system, no conformation may be deformed into its mirror image This is reflected in the MS group of the molecule, which is dissymmetric The chirality of **74** is demonstrated experimentally by NMR experiments done in the presence of the Pirkle chiral solvating agent [34b] But, is the molecular graph of **74** topologically chiral? This question is not nearly so easy to answer, since it is impossible to examine every possible *presentation* of a construction Indeed, none of the conventional elements of topological dissymmetry (chiral link or knot) are present in this construction A discussion of the novel topology of graphs exemplified by **74** is given in the next section

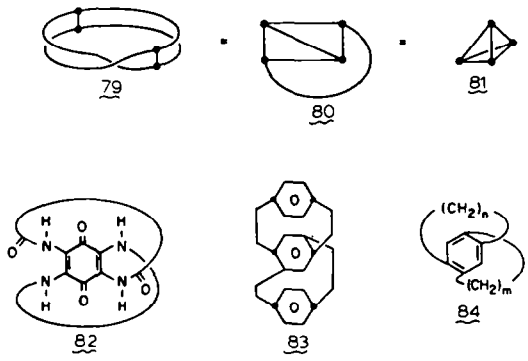
3 *Topology of the molecular Mobius strip the Mobius ladders* A Mobius strip is a two-dimensional object a surface The intrinsic topological properties of a Mobius strip are well known They include the one-sidedness of the strip (topologists say the surface is non-orientable), and the fact that it remains in one piece when cut "in half" The well-known chirality of a Mobius strip is an extrinsic property of embedding the strip in 3-space Intrinsically, a Möbius strip is homeomorphic to any strip with an odd number of half-twists of either handedness [10]

Of course, the molecular graph of the tris-THYME Mobius strip **74** is a one-dimensional construction, not a surface This graph does, however, possess many of the most interesting topological properties of a Mobius strip Graphs of type **74** have been described in the mathematical literature Appropriately, this graph is termed a *Mobius ladder* [43] Harary and Guy define the Möbius ladders as the $n$-gons ($n > 5$, and even) and all edges joining opposite vertices Standard presentations of the Mobius ladders with $n = 6$ and 8, termed $M_6$ and $M_8$, respectively, are shown in Scheme 25 Intrinsically, as mentioned above, the normalization of the crossings is meaningless Scheme 26 shows presentations of $M_6$ (**77**) and $M_8$ (**78**) embedded in a special way in 3-space The origin of the term Mobius ladder is clear from these presentations Note that $M_6$ is isotopic with $K_{3,3}$, the second Kuratowski non-planar graph In drawing **77** the vertices are differentiated such that the "houses" ($\times$'s) and "utilities" (○'s) are easily seen In fact, all of the Mobius ladders are minimally non-planar, and possess $K_{3,3}$ as a subgraph Thus, one necessary condition of topological chirality is fulfilled for the molecular Möbius strip **74**, namely non-planarity of the molecular graph

An interesting result concerning molecular Mobius strips of type **74** may be mentioned at this point Specifically, one may readily imagine synthesis of a "Mobius ladder" with only two rungs (**79**), as shown in Scheme 27 Indeed, such syntheses have been carried out In 1971 Schill reported preparation of a two-rung Mobius ladder, the quinone **82**,[44] in connection with his work directed towards the synthesis of a knotted ring At the same time, Nakazaki prepared several such two-rung ladders, including **83** and **84**, in connection with his work on cyclophane chemistry [45] All of these novel compounds have molecular graphs equivalent to **79** The Euclidean geometry of the [$n$],[$m$]-cyclophanes **84** are closely related to the betweenanenes of Marshall,[46] though the latter materials have topologically different molecular graphs

Intuition would suggest that a two-rung ladder of type **79** would have topological properties similar to those of $M_6$ (**77**) But, four vertices are not sufficient to define a non-planar graph Indeed, none of these novel compounds **82–84** is a Mobius ladder by the mathematical definition, and they are all in essence isotopic with the planar presentation **80** Interestingly, the two-rung ladder **80** is also isotopic to the tetrahedral graph **81** Thus, the graph **79**, and compounds **82–84**, cannot be topologically chiral


Scheme 26 Alternative presentations of $M_6$ and $M_8$



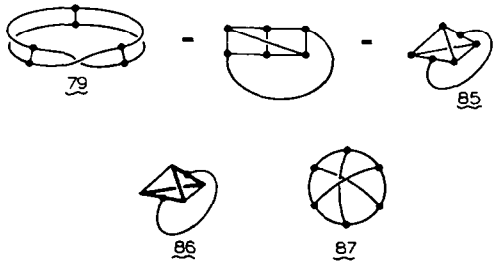

Scheme 27 The "two-rung Mobius ladder"

This fact translates into chemical reality For example, if structure **79** represented an enantiomerically pure bis-THYME polyether, then the molecule could racemize by rotation about the THYME double bonds (a "*cis–trans*" isomerization) Such a racemization is impossible for tris-THYME Möbius strip **74**

4 *Topological chirality of the Möbius ladder with three differentiated rungs* While the molecular Mobius strip **74** is clearly non-planar, it seems reasonable that non-planarity in itself is not sufficient for topological chirality Indeed, as shown in Scheme 28, presentation **85** of a Möbius ladder is rigidly achiral! Therefore, a Mobius ladder with nine equivalent edges and six equivalent vertices is *topologically achiral* In fact, both $K_{3,3}$ and $K_5$ with identical vertices and edges are topologically achiral Of course, the molecular Mobius strip **74** has two kinds of edges, the double bonds and the ethyleneoxy chains Drawing **86** reproduces the achiral presentation **85**, but differentiates the double bonds (light edges) from the ethyleneoxy chains (dark edges) This differentiation clearly destroys the reflection symmetry of **85** To our knowledge, the highest symmetry attainable for a model of **74** is represented by presentation **87** This presentation has $D_2$ symmetry and is therefore chiral

The preceding discussion illustrates the way in which most chemists would go about trying to establish the chirality of a molecular graph That is, the first step is to search for a rigidly achiral conformation If such a conformation is found, chirality of the molecule is ruled out But, if no such conformation exists, then is chirality of the molecule assured? In fact, the answer to this question is no Consider removing a right-handed rubber glove from your right hand by "peeling" it off, and in the process turning it inside-out The resulting object is superimposable on a left-handed glove, yet at no time did the glove ever attain a rigidly achiral "conformation" [21] An exactly analogous scenario exists in chemistry Specifically, in 1954 Mislow first described a class of meso compounds for which individual molecules can never achieve a rigidly achiral conformation, and later synthesized the first example [12b,47]

As shown in Scheme 29, the prototypal example of this type of achiral species is the biphenyl **88** Rotation about the central bond is sterically disallowed at room temperature But, rotation about the bonds at the p-p′ positions of the biphenyl are unhindered Given these constraints, molecules of type **88** can never achieve a single rigid conformation with reflection symmetry That is, all possible conformations are chiral Yet, simple rotation about the unhindered p-p′ bonds allows interconversion between mirror-image conformations, as shown in the scheme This is analogous to the




Scheme 28 The three-rung ladder

88

Scheme 29 An achiral dissymmetric molecule

"racemization" of gauche-butane conformations or interconversion of chiral meso-tartaric acid conformations The difference is that with gauche butane or meso-tartaric acid, at least one rigidly achiral conformation is accessible, while biphenyl **88** has none

The latter type of achirality is quite rare, but does exist in other systems, including enantiomerization of phosphoranes by the Berry pseudorotation mechanism[48] and in two other novel systems designed by Mislow enantiomerization of maximally labeled triarylamines by the two-ring flip mechanism[49] and enantiomerization in certain geared bis(9-triptycyl)methane derivatives [50] The achirality of compounds of type **88** is reflected in the non-rigid MS groups of such compounds, which are nondissymmetric

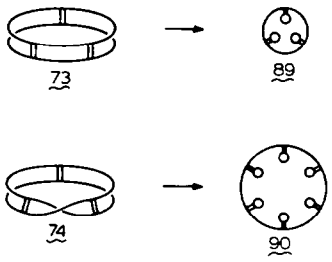
For a rubber glove, or for biphenyls of type **88**, a rigid achiral *presentation* is accessible topologically A glove may be deformed into a planar disk, while the biphenyls **88** may attain an achiral presentation if the aryl rings are made coplanar Is the above described sort of achirality possible topologically? This is indeed a fascinating topological question which is suggested in a straightforward way by consideration of the topology of molecular graphs from a chemists perspective One way of posing this question as it relates to the molecular Mobius strip **74** is the following if it could be shown that there is no rigidly achiral presentation of structure **74**, is topological chirality assured? A more general way of asking the question is does there exist any one-dimensional construction embedded in 3-space which is rigidly chiral in every presentation, but topologically achiral? To our knowledge, the answer to this interesting problem is not known [51]

Is the molecular Mobius strip topologically chiral? As discussed above, even if presentation **87** were the highest symmetry presentation possible for the molecular graph of **74**, it is not known whether topological chirality would be proved Of course, it is impossible to know if all non-assymmetric presentations have, in fact, been examined While with models we have never succeeded in interconverting mirror images of **74**, such negative experimental evidence must be considered very cautiously, since such experiments are surprisingly difficult to do The only way to really know that a molecule is topologically chiral is to prove it mathematically Generally this means finding a chiral knot or link in the construction The molecular graph of **74** has neither Surprisingly, to our knowledge a proof of the chirality of a Möbius ladder with three differentiated rungs was not available in the mathematical literature until after the molecule was synthesized Just this year, after discussing the problem with the author, a proof of the topological chirality of such graphs has been produced by Jonathan Simon of the Department of Mathematics at the University of Iowa [52] Thus, with a proof in hand, we can say that (1) there is no rigidly achiral presentation of the graph of **74**, and (2) the graph cannot be converted into its mirror image in 3-space by continuous deformation

5 *Breaking the rungs of a Mobius ladder* As mentioned above, when cut in "half", a Möbius strip remains in one piece The molecular Möbius strip **74** possesses this property also Intrinsically, cutting a Möbius band in half results in a single *orientable* surface One may demonstrate experimentally that cutting a Mobius strip embedded in 3-space in "half" results in formation of a single band with four half twists Any band with an even number of half twists has two edges and two sides, and is an orientable surface Interestingly, cutting the non-orientable Mobius strip gives an orientable product It is impossible, however, for such a cutting of an orientable surface to produce a non-orientable one

The molecular Möbius strip defines a Mobius ladder, as discussed above The "rungs" of the ladder may be considered as embedded in a Mobius band, while the edge of the ladder forms the boundary of the Mobius surface Thus, breaking the rungs of the ladder is equivalent to cutting a Möbius strip in "half" With the molecular Mobius strip **74**, the THYME double bonds are the "rungs", while the ethyleneoxy chains make up the edge A chemical cutting of the Möbius strip simply involves cleavage of double bonds

Originally, this cleavage reaction was envisioned as an important method for distinguishing between **74**, and the cylindrical isomer **73** Actually, the structural problem was readily solved by X-ray and NMR techniques The "clipping reaction", however, is still a novel process, and necessary if catenanes and knots are to be synthesized by this strategy After considerable experimentation on




Scheme 30 The clipping reaction

model systems, good evidence showing the workability of such a process has been obtained As shown in Scheme 30, application of a selective ozonolysis procedure to the cylinder **73** and the Mobius molecule **74**, gives products tentatively identified as the novel crown ethers **89** and **90**, respectively These products have been characterized by TLC, analytical gel permeation chromatography, $^{1}$H- and $^{13}$C-NMR spectroscopy, IR, and FAB mass spectrometry If these structures prove correct, the way will be clear for accomplishment of a synthesis of a molecular knotted ring by the Mobius strip approach We feel the trefoil knot is really the "classic" problem in topological stereochemistry Therefore, a discussion of the approach, and the topology of the two-braid products, is given below

C *Synthesis of molecular knots*

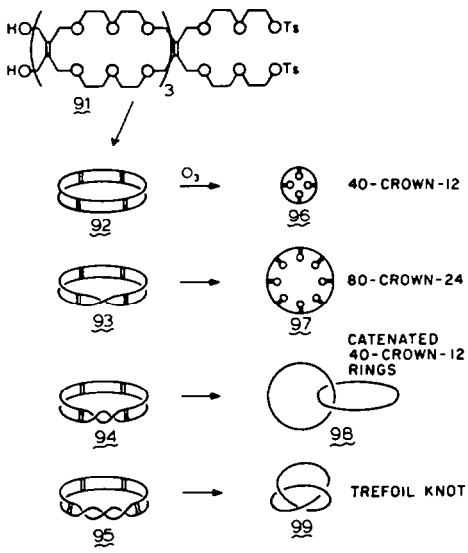
1 *Introduction* Philosophers and scientists have historically delighted in devising explanations of nature based upon geometrically interesting constructions In chemistry, this pursuit dates back at least to Plato, who developed a theory of the interconversions of matter based upon the Platonic solids [1] Knotted rings also have an interesting history related to chemistry Such prestigious scientists as Helmholtz and Lord Kelvin believed that the structure of atoms was somehow related to knotted "threads" They proposed that different knottings of a closed curve were responsible for the different elements Hydrogen was thus a simple unknotted ring, and it was even suggested that the structure of the $H_2$ molecule involved two linked rings Of course, such ideas have not survived the test of time Nevertheless, they served an important function because these theories were the rationale for one of the first attempts to mathematically classify knots Tait, one of the originators of knot theory, was encouraged by Lord Kelvin in the hopes that classification of knots would somehow help in understanding the structure of the elements [30] While not so fundamentally important in chemistry and physics as Kelvin had originally hoped, knot theory is certainly an important mathematical pursuit today

More recently, the pristine geometry of the tetrahedron, cube, and pentagonal dodecahedron certainly helped define several very challenging targets for directed total synthesis Indeed, the effort expended towards achieving the sculpturing of graphs corresponding to these solids in three-dimensional hydrocarbon networks has been monumental The rewards, however, have been great, culminating most recently in Paquette's total synthesis of dodecahedrane in 1982 [53]

Chemical synthesis of molecular knotted rings is also an interesting challenge To our knowledge, outside of the DNAs, no molecular knot has ever been characterized Difficulties with the approach involving random knotting of a single functionalized chain were discussed above The Mobius strip approach, however, appears to afford a much more elegant route to a knotted ring In addition, several other topologically novel constructions result from application of the approach to synthesis of a knot

2 *Synthesis of a molecular trefoil knot and the topology of the higher Mobius ladders and chiral prisms* The Möbius strip approach to synthesis of a trefoil knot is outlined in Scheme 31 in the context of the THYME strategy Examination of space-filling models indicates that the tetra-THYME diol-ditosylate **91**, possessing two 40-atom edges, should allow the degree of twisting necessary to obtain a knot Thus, upon treatment with base under conditions of high dilution, diol-ditosylate **91** could give four diastereomeric products tetra-THYME cylinder **92** with two 40-atom edges, Mobius strip **93** with a single 80-atom edge, cylinder **94** with two half twists, and Möbius strip **95**, with three half twists The latter product possesses a knotted subgraph More highly twisted isomers appear disfavored sterically

The molecular graph of a tetra-THYME cylinder **92** is a prism, while isomer **93** is, of course, the Mobius ladder $M_8$ The prism **92** is achiral, and is shown in a rigid achiral presentation in the scheme It is expected that the allylic methylene signal for **92** will be a singlet at room temperature, since in this




Scheme 31 The THYME approach to synthesis of a trefoil knot

system the "inside out" conformational deformation should be facile As discussed above, the Mobius ladder **93** is clearly topologically chiral, possessing the structure **74** as a subgraph But, the topology of the graph **93** differs from that of **74** in an interesting way We conjecture that this four-rung Mobius ladder is topologically chiral even ignoring the differentiation of the rungs Thus, even assuming eight identical vertices, and twelve identical edges, this graph may be topologically chiral Certainly within the context of the structures shown in Scheme 28 there is no rigid achiral presentation of the Möbius ladder $M_8$

The twisted prism **94** also has novel topology Prism **94** possesses no Kuratowski non-planar subgraph In addition, no oriented link, and no knotted circuit is present But, **94** is minimally non-planar by virtue of a link subgraph, and we conjecture that this prism is also topologically chiral Finally, graph **95** is homeomorphic with $M_8$ and is certainly topologically chiral, possessing a trefoil knot subgraph The surface defined by this construction, a three-half-twist Mobius strip, has considerable esthetic appeal in some presentations—examine the Pure Wool label on a woolen article of clothing Note that **92** and **94** are *topological diastereomers*, as are **93** and **95**

Cleavage of the rungs of these four constructions would produce the crown ether products **96–99**, as shown in the scheme Our proposal for proof of the structures of these eight novel compounds relates to the topology of the systems, and is therefore outlined here This plan, of course, rests entirely on our ability to obtain each of the four THYME wreaths in pure form

Of the four pentacyclic products **92–95**, only cylinder **92** is achiral This product may in principle be distinguished from the other three by $^{13}$C-NMR in the presence of a chiral solvating agent The structure proofs envisioned for the remaining wreaths require the clipping reaction Ozonolysis of compounds **92–95** will afford the crown ethers **96–99** For crowns **96–98**, the protons of the methylene groups $\alpha$ to the carbonyls are enantiotopic, and will appear as a sharp singlet in the $^1$H-NMR spectrum, as is observed for crown ethers **89** and **90** in Scheme 30 The molecular trefoil knot **99**, however, is topologically chiral The methylene protons $\alpha$ to the carbonyls of this molecule are diastereotopic, and should appear as an AB quartet In addition, since compound **99** is a racemate, two carbonyl carbons should appear in the $^{13}$C-NMR of **99** in the presence of a chiral solvating agent, and chromatography on a chiral solid phase may allow resolution of the racemate Any of these observations will serve to differentiate knotted ring **99** from crowns **96–98** thereby proving the structures of the three-half-twist wreath **95**, and the molecular trefoil knot **99** We envision mass spectrometry, e g FAB mass spectrometry with collision induced fragmentation, to distinguish between crown ethers **96**, **97**, and **98** The diagnostic mass spectra of catenanes have been discussed [2c] Note that compounds **97** and **99** exhibit the prototypal topological diastereoisomerism

The molecular trefoil knot **99** is especially interesting since here chemical reality approaches the topological model Chemically, an 80-membered ring of atoms all joined by single bonds is "completely flexible" This molecule has no stereocenters and no other molecular rigidity Yet, trefoil **99** is chiral, and

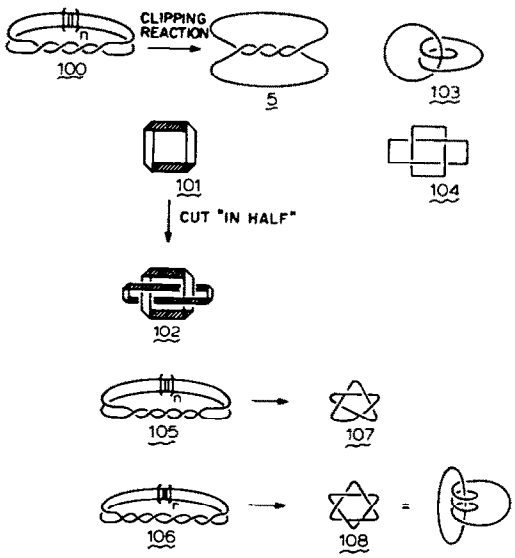
a diastereomer of unknotted ring **97** It may be truly stated that the trefoil knot **99** is chiral solely by virtue of its topology Such a statement seems incorrect for the Mobius strip **74**, possessing as it does considerable Euclidean rigidity

The higher two-braid knots and links are also quite interesting, topologically and esthetically Examples up to minimum crossing number 6, at least in principle obtainable chemically by the Mobius strip approach, are shown in Scheme 32 The four-half-twist prism **100** has special significance in that the surface defined by this construction results upon cutting a Möbius band in half Also, as for the Mobius band, the four-half-twist strip has considerable esthetic appeal, particularly in the presentation **101**, in which each half twist is put at the corner of a square This appeal is documented by the rather large number of organizations utilizing this, or a similar construction, as their logo Cutting strip **101** in half (a second cutting of a Möbius strip) affords two linked strips, each with four half twists! Drawing **102** shows an interesting representation of this construction which may easily be prepared with paper and scissors Of course, breaking the rungs of prism **100** gives the link with four crossings This link is shown in three common presentations the two-braid link **5**, the "double-looped catenane" **103**, and the link **104** (reproduced from Scheme 18) Chemically, the double-looped catenane has considerable significance, representing a topologically chiral catenane Such a construction has never been isolated, and represents another classic unsolved problem in topological stereochemistry

The products of clipping of five and six half twisted Mobius ladders and prisms (**105** and **106**, respectively), are shown in their well-known presentations **107** and **108** Construction **107** is the five-star knot, also called a Simony knot after the first person to describe it [3] There are two knots with a minimum of five crossings Only the five-star **107**, though, is a two-braid The actual Mogen Dovid (shield of David) of Biblical times was usually drawn as two interlocked triangles showing the normalization of the crossings, as in structure **108** All of these constructions are well known to be topologically chiral Concerning the prospects for actual synthesis and characterization of such compounds, the system with five crossings becomes problematical, since it is difficult, without crystals, to envision an unequivocal proof of structure of a five-star macrocycle relative to its trefoil isomer Of course these compounds could be crystalline, and the allure of attempting syntheses along the lines shown in Scheme 32 seems impossible to resist

3 *The three-braids* Possibilities for the synthesis of several other special topological constructions have been discussed in the literature [2 21] Specifically, several members of a class of structures which may be termed *three-braids* are especially intriguing and are discussed below

With a "twisted cylindrical" paper band with some number of half twists, it is possible to effect a "trisection" of the band by starting to cut one-third of the way in from an edge, and continuing until that initial cut is reached again Often, the results of such a cutting of an actual paper strip are surprising Thus, trisection of a paper Mobius strip gives linked strips, one of which is half the diameter of the other




Scheme 32 Wreaths with four, five, and six half twists

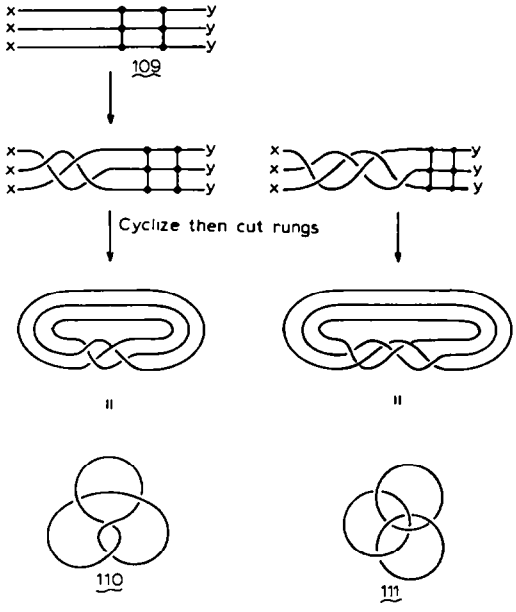

Scheme 33 Some three-braids.

The smaller strip is a Möbius strip, and the larger is a strip with four half twists Amazingly, it is possible to arrange this construction in the form of a three-layered "Mobius strip" with the larger strip comprising the "top" and "bottom" layers, and the smaller Möbius strip sandwiched in between! Cutting of other more highly twisted bands also give very interesting results As for the bisection of the two-braid Mobius ladders and chiral prisms, cutting the rungs of three-stranded ladders (three-braids) gives products which may be termed the *three-braid knots and links* The products resulting upon trisection of three-stranded ladders cyclized with $n$ half twists ($n = 1$–5) are shown in Scheme 33 [21]

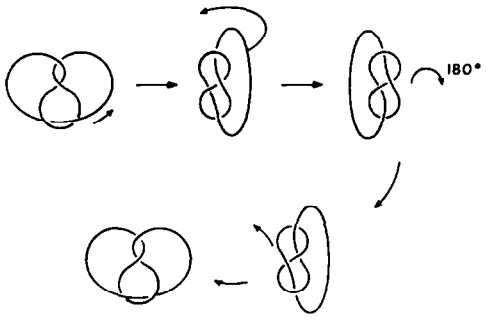
For the three-stranded ladders, alternative modes of cyclization can give some even more novel constructions Specifically, as shown in Scheme 34, a three-stranded ladder exemplified by construction **109** with two rungs, may in principle cyclize to afford the figure-of-eight knot (**110**), and the Borromean rings (**111**) These constructions are among the most topologically interesting and famous of the three-braid knots and links, and a short discussion of these intriguing structures is presented

The figure-of-eight knot is the only knot with a minimum of four crossings That the figure 8 is topologically achiral is easily demonstrated by determination of the configuration of the four crossings, as discussed below Interestingly, a "chiral pathway for racemization" is possible for the figure 8 as shown in Scheme 35 A rigidly achiral presentation with an $S_4$ axis of symmetry is shown in Scheme 36 We conjecture that the figure of eight is one of the knots, known to exist,[51] which is achiral but has no presentation with a mirror plane We also conjecture that no symmetry presentation of the figure-of-eight knot has the minimum of four crossings

The Borromean rings **111** were used as the coat of arms of the famous Italian Renaissance family Borromeai, and is a popular symbol in Italy today [3 21] It was also the logo of the Ballantine Beer Brewery (though in about 1955 Ballantine stopped showing the crossings on their labels[21]) and of the Krupp works in Germany Note that *all three rings are linked, but no two are linked*! Thus, cleavage of one ring results topologically in a line, and two *unlinked* rings In general, *any number of rings* can be linked with no two rings linked [3] The Borromean rings are also topologically achiral




Scheme 34 The figure-of-eight knot and the Borromean rings




Scheme 35 A chiral pathway for "racemization" of the figure-of-eight knot

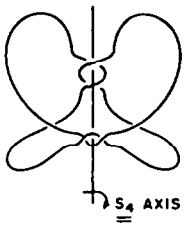
D *Catenanes by olefin metathesis a topological alternative to the Mobius strip approach*

In 1968 Wasserman at Bell Labs reported evidence for the formation of very large ring hydrocarbons (up to 120 carbons) by olefin metathesis of cyclooctene [54] Based upon mechanistic considerations, it was suggested that catenanes and knots should also be produced in this reaction Later, Wasserman, and Wolovsky in Israel reported that, based upon mass spectral evidence, large amounts of catenanes were indeed formed in the olefin metathesis reaction of macrocyclic polyenes [55] The initial rationalization of these interesting results involved a pairwise intramolecular metathesis process as illustrated in Scheme 37 for a macrocyclic tetraene (**112**) Thus, pairwise interaction of two double bonds of the polyene was thought to give rise to cylindrical intermediates of type **113** and to twisted intermediates like **114** Metathesis would then lead to formation of catenanes and knots in a manner exactly analogous to the Möbius strip approach to formation of the two-braids

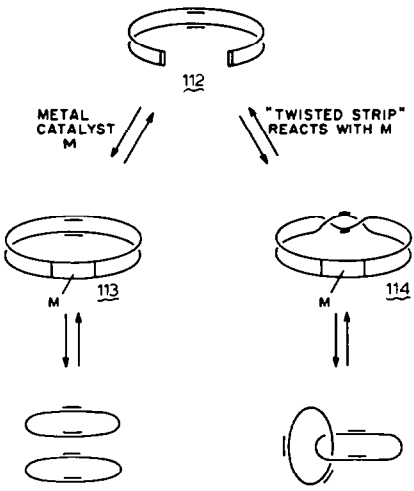
Since then, however, there seems to be a consensus among organometallic chemists that the metathesis reaction in fact proceeds by an alternative mechanism involving the formation of metal alkylidene and metallacyclobutane intermediates [56] The key intermediate in the intramolecular metathesis reaction of a macrocyclic tetraene of type **112** would then be the acyclic alkylidene **115**, as shown in Scheme 38 How then, does one account for the formation of catenanes in these reactions without requiring a threading process—known to be quite disfavored?

One possible answer to this topological puzzle is shown in Scheme 38 [57] It seems quite likely that the acyclic chain may adopt a conformation possessing a twisted loop as shown in structure **116** No threading is required to get to this conformation If the carbene inserts into a double bond in the loop, the construction **118** is produced, via the metallacyclobutane intermediate **117** Thus, a threaded macrocyclic ring is produced without requiring an intra- or intermolecular threading process If the metathesis reaction is fast compared to the rate at which threading equilibrium is established, i e if a second metathesis of **118** proceeds faster than "unthreading", the link **119** would be produced, and the metal alkylidene catalyst regenerated

It seems reasonable that this scenario would predict very large quantities of linked rings compared to a random threading approach A triple-twist intermediate similar to **116** would give the trefoil knotted ring, etc Indeed, this process is operationally equivalent to the Möbius strip approach, and affords all of the two-braid knots and links, though no Mobius ladder intermediates are involved [57] The two operations proceed to give the same products, but the topology of the intermediates differ While the actual mechanism by which links are generated in these interesting metathesis reactions remains to




Scheme 36 A rigidly achiral presentation of the figure-of-eight knot




Scheme 37 Catenanes by olefin metathesis—first proposal

be elucidated, the above explanation seems satisfactory Further work on isolation of the products of the metathesis reactions, including work on isolation of a knotted hydrocarbon ring produced by olefin metathesis, are under way in the laboratories of E Wasserman at DuPont

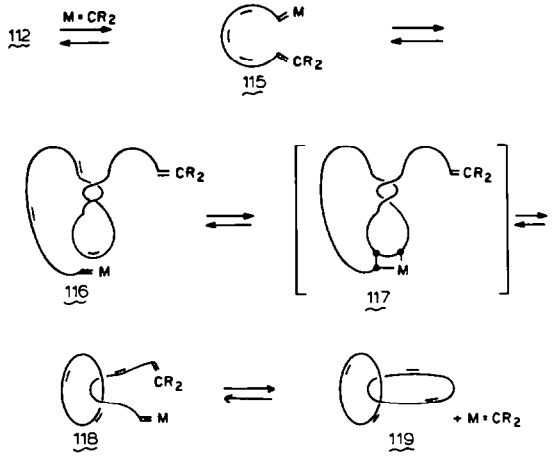
## PART IV OTHER TOPOLOGICAL STEREOISOMERISM

A *Other stereoisomers with oriented rings as elements of dissymmetry the Prelog cyclostereoisomers*

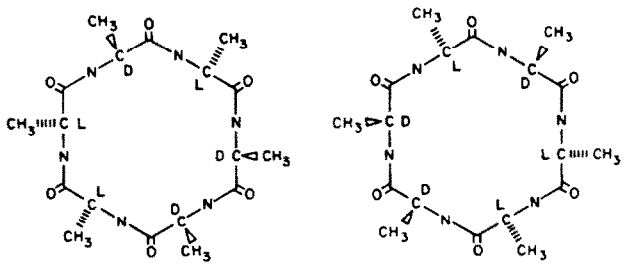
While they are not topological stereoisomers by our definition, the interesting cyclostereoisomers of Prelog possess one topological contribution to their chirality and should be mentioned in this report In this case, a single oriented ring possesses conventional chirality about its periphery If the substitution pattern is correct, enantiomers or diastereomers may exist for which one element of the stereoisomerism is topological and the other elements are Euclidean Prelog first described such novel stereoisomers, and coined the term cyclostereoisomers to denote them [58] A pair of cycloenantiomeric cyclic peptides synthesized in Prelog's laboratory is shown in Scheme 39 It seems possible that examples of this type of stereoisomerism also exist in the natural product literature Note that, for example, any macrocyclic lactone defines an oriented circuit

B *Other non-planar molecules*

1 *Introduction* Of all the interesting, highly connected and novel molecules which have been isolated or synthesized over the last 200 years, molecules possessing topologically non-planar




Scheme 38 A topological alternative to the Möbius strip approach

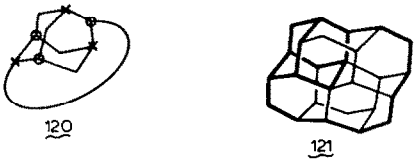


Scheme 39 Cycloenantiomers

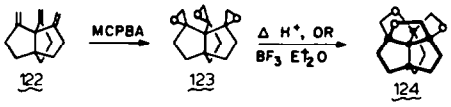
molecular graphs are remarkably absent This fact has certainly intrigued chemists for many years In a proposal from the R B Woodward group (written mainly by Howard Simmons III) submitted to the NSF in 1978, Woodward wrote that he "was asked in 1948, by James K Senior (Chicago) whether any such substance was known, then as now, the reply was in the negative" [59] The latter statement is no longer true. But, molecules with non-planar molecular graphs are a very recent development This is especially surprising given that the vertices of the $K_5$ graph are "tetravalent", and those of the $K_{3,3}$ graph are "trivalent" It is not required that a non-planar graph possess just one crossing It may be a highly interconnected network It seems very surprising to this author that no naturally occurring material isolated to date has a non-planar molecular graph!

The "difficulty" in attaining non-planarity of molecular graphs is nicely illustrated by consideration of the adamantanes It is intuitively reasonable that a diamond must have a non-planar structure Indeed, this is true Any three-dimensional network will have a non-planar subgraph if it is large enough One may ask, then, how many adamantane units (polymantanes come in units of $C_{4n+6}H_{4n+12}$) are required for non-planarity? Consider a bridged adamantane as shown in structure **120** (Scheme 40) This construction indeed possesses a $K_{3,3}$ subgraph The vertices of the non-planar subgraph are labeled with ×'s and ○'s [60] A molecule with structure of type **120** would be similar in some interesting respects to the class of paddlanes,[61] and does not, to our knowledge, exist How many adamantane units must be added to achieve the joining of two opposite secondary carbons of adamantane as represented in structure **120**? Surprisingly, it is conjectured that at least four additional "adamantane units" are required Thus, the smallest non-planar polymantane is the pentamantane isomer **121** ($C_{26}H_{32}$) One $K_{3,3}$ subgraph of **121** is outlined in bold To our knowledge, no pentamantane isomer has yet been isolated [62]

2 *The* $K_5$ *molecules* Considering only molecules with no metal atoms, the first synthesis of a Kuratowski non-planar molecule was accomplished in 1981 Synthesis of the interesting $K_5$ propellane derivative **124** (Scheme 41) was published simultaneously by Howard Simmons III working in Woodward's group,[63] and by Leo Paquette [64] As shown in Scheme 41, treatment of 2,8,9-trimethylene[3 3 3]propellane **122** with MCPBA gave two separable diastereomeric tri-epoxides **123** Treatment of either tri-epoxide with a sulfonic acid ion exchange resin, Lewis acid, or heat afforded the topologically non-planar propellane derivative **124** The molecular graph of **124** is a beautiful example of a $K_5$ non-planar graph The vertices of the $K_5$ graph are emboldened in the drawing of **124** As pointed out above, if the edges joining these five vertices were identical, the molecule would be topologically achiral But, the molecular graph of **124** has a much more complex topology The $K_5$ graph represented by structure **124** has three kinds of edges, C—C single bonds, —$CH_2CH_2$— chains, and —$CH_2O$— chains The latter are oriented edges We conjecture that structure **124** is topologically chiral If this conjecture proves correct, then to our knowledge, **124** would be the first topologically chiral non-polymeric organic molecule ever isolated



Scheme 40 A non-planar polymantane




Scheme 41 The Simmons–Paquette $K_5$ molecule

Clearly, organometallic compounds and metal clusters provide ample opportunity for topological non-planarity Indeed, in many metal cluster systems the pairwise bonding formalism becomes unrealistic, and one may consider all the metal atoms in the cluster bonded to every other atom Since 1977 several formally non-planar metal clusters have been characterized [65] Apparently, however, there are no topologically chiral clusters

To our knowledge, the first examples of topological chirality to appear in the chemical literature involved a novel class of ferrocene derivatives, as shown in Scheme 42 In 1977 the first of a novel class of chiral ferroceneophanes exemplified by structures **125** and **126** was synthesized in the laboratories of Koji Yamakawa [66] The molecular graphs of these molecules may be reduced to $K_5$ Again, it is conjectured that the molecular graphs of **125** and **126** are topologically chiral, though a proof is not in hand

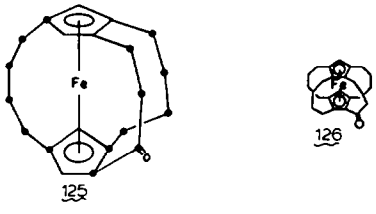
## PART V SPECIFICATION OF CONFIGURATION FOR TOPOLOGICAL STEREOISOMERS

Specification of configuration of molecules independent of conformation is, of course, an important aspect of the nomenclature In organic and inorganic chemistry very useful and well-known methods have been developed to accomplish this task The problem of assignment of configuration for topological stereoisomers, however, is a very interesting one which actually interfaces with a very well-known problem of low-dimensional topology While no comprehensive solution to the chemical nomenclature problem will be presented here, a short discussion of the special character of configuration assignment for topological stereoisomers is given, along with some suggestions for how this assignment may be accomplished in certain cases

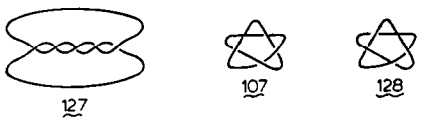
Methods for assignment of configuration of catenanes with conventional Euclidean elements of chirality in the rings, and for knotted molecular rings, have been suggested in the literature [67] But, as pointed out recently by Damhus and Schaffer, none of the currently used methods for specification of chirality, such as the well-known *R,S*-descriptors of Cahn, Ingold, and Prelog[68] is applicable in any obvious way to specification of topological chirality of molecules such as the molecular Mobius strip **74** [69] Such a method would be especially useful, however

A simple example best serves to illustrate this point As indicated by the discussion on the higher Mobius ladders given above, the two-braid knot with five crossings (**127**, Scheme 43) is isotopic with the five-star knot But, the five star is chiral, and exists as the pair of non-isotopic enantiomeric structures **107** and **128** How can one tell whether the two-braid **127** is isotopic with five-star **107** or with **128**? One obvious solution would be to make structure **127** from a piece of rope, then to deform it into a five star and simply identify the result with **107** or **128** But, is there a way of accomplishing this identification without actually interconverting the isotopic constructions either mentally, or with a physical model?

If one could devise a method for *assignment of configuration* to the knots shown in Scheme 43, independent of the presentation, a ready solution to this question would be in hand This particular topological question is a simple example of an *isotopy problem*, and is easily solved as described below Interestingly, for the general case it is very difficult to assign configuration completely independently of




Scheme 42 Topologically chiral ferroceneophanes




Scheme 43 Illustration of a simple isotopy problem

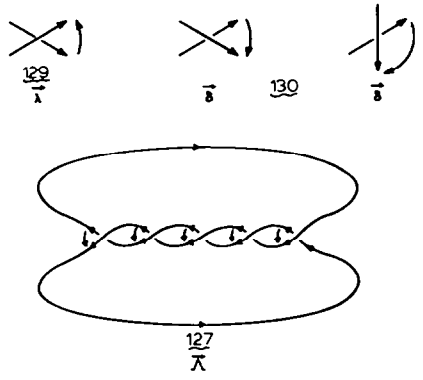
presentation Indeed, it is difficult in general to establish isotopy between different knots, let alone different enantiomers For example, suppose one had a trefoil knot made of a long circle of string The string could be "balled up" such that a planar projection of the resulting presentation would have literally thousands of crossings It is a very deep topological problem to derive, from properties of that presentation alone, whether the construction is isotopic with a "right-handed" trefoil knot, or its mirror image, or indeed with a five star, an unknotted ring, or any other knot The most workable solution is the trivial one—that is to "untangle" the string until a recognizable presentation is reached

There is, however, a very easy way to establish isotopy of two-braid knots, that is specify configuration, *if the presentations in question have the minimum number of crossings* For this much simplified case, the *oriented skew lines* convention, as proposed by Damhus and Schaffer, works well to specify chirality, and thus to allow solution to isotopy problems such as that shown in Scheme 43 [67,69]

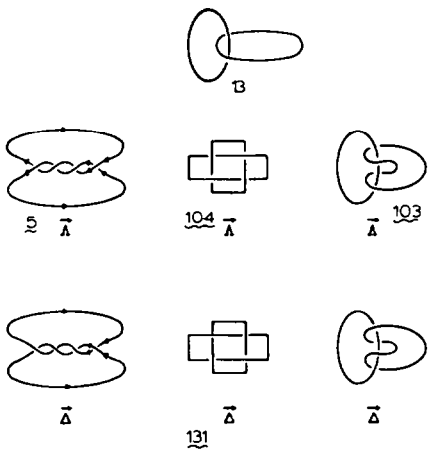
For specification of chirality, some chiral reference system is required The chiral reference system used here is the pair of oriented skew lines, as shown in Scheme 44 [67] One line passes over the other, and both are oriented Such a construction is chiral unless the lines are parallel or antiparallel Thus, mirror image constructions **129** and **130** are distinct if the lines are never allowed to become parallel or antiparallel Chirality descriptors are assigned as shown **129** is $\vec{\lambda}$, and **130** is $\vec{\delta}$ We find the following device most useful for allowing easy assignment of configuration to oriented skew lines If, when tracing a path from the "arrow head" of the bottom line segment to the "arrow head" of the top line segment without crossing either line, a clockwise motion (projected in the plane) results, then the system is $\vec{\delta}$ Similarly, if a counterclockwise arc results upon going from the bottom "arrow head" to top, the system is $\vec{\lambda}$ These descriptors are independent of presentation so long as the lines are not allowed to become parallel or antiparallel, as illustrated for the $\vec{\delta}$ oriented skew lines **130**

Application of this convention for specification of configuration of knots is straightforward First, the knot must be in a presentation with the minimum crossing number The chirality descriptor will be independent of presentation within this limit The knot is then given an arbitrary orientation It makes no difference in the final result which direction is chosen At each crossing, a determination is made whether that *crossing* is $\vec{\delta}$ or $\vec{\lambda}$ Application of the oriented skew lines convention in this case is quite straightforward The number of $\delta$s and $\vec{\lambda}$s are then summed arithmetically If there are the same number of $\vec{\delta}$ and $\vec{\lambda}$ crossings, then the knot must be topologically achiral If there are more $\vec{\lambda}$ crossings, the knot has configuration $\vec{\Lambda}$ If there are more $\vec{\delta}$ crossings, the knot is $\vec{\Delta}$

Thus, the two braid with five crossings shown in Scheme 43 (**127**) and redrawn in Scheme 44, has five $\vec{\lambda}$ crossings, and zero $\vec{\delta}$ crossings, and is therefore assigned the $\vec{\Lambda}$ configuration As the reader can easily demonstrate, five-star **107** is $\vec{\Delta}$, while **128** is $\vec{\Lambda}$ Thus, two-braid **127** is isotopic with five-star **128** It is satisfying to note that in the two-braid presentation, this braid has left-handed helical chirality For any




Scheme 44 The oriented skew lines convention for knots




Scheme 45 Specification of configuration for the two-braid links

two-braid knot in a wreath-like presentation, the $\vec{\Delta}$ knot will have right-handed helical chirality in the braid, and the $\vec{\Lambda}$ knot will be a left-handed helical braid

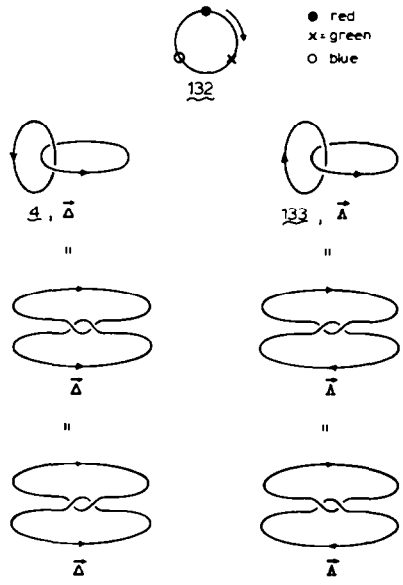
Application of the skew lines convention to *links* is less straightforward Consider the case of the two-braid links The achiral two-braid with two crossings (**13**), and the two enantiomeric two-braids with four crossings (**5** and **131**, shown in several presentations), are shown in Scheme 45 First, note that arbitrary assignment of orientations to the rings of the link **13** may be accomplished in two different ways Application of the rules described above for assignment of configuration at each crossing results in either two $\vec{\lambda}$ or two $\vec{\delta}$ crossings for **13** depending upon how the orientations were assigned Thus, the achirality of the link **13** is not indicated by equal numbers of $\vec{\lambda}$ and $\vec{\delta}$ crossings as is the case for the two-braid knots

For chiral links such as **5** and **131**, another convention is required in order to allow unambiguous assignment of configuration based on the $\vec{\delta}/\vec{\lambda}$ rules, since again arbitrary assignment of orientation to the rings of the link may be done in two ways We suggest the following convention For a two-braid link with four or more crossings, the rings are oriented *parallel* Application of the rules described above for knots then allows assignment of configuration to the link The parallel orientation is that in which the arrows are pointing in the same direction in the braided part of a standard two-braid presentation, as shown in the scheme The direction of the parallel orientation may be arbitrarily chosen Each crossing is then assigned a $\vec{\lambda}$ or $\vec{\delta}$, and the configuration of the link is established as for the two-braid knots Thus, link **5** is $\vec{\Lambda}$, and link **131** is $\vec{\Delta}$ If this convention is utilized, then the $\vec{\Delta}$ link in the two-braid presentation is a right-handed helix

Of course, to be most useful, the method for assignment of configuration should work in any presentation with a minimum crossing number We conjecture that the above described method fulfills this criterion, if the following definition of parallel orientation is utilized Two "adjacent" crossings of the construction are picked The arcs between these crossings are then oriented such that both arrows point from one crossing to the other This is a parallel orientation Application of the rules will then allow assignment of configuration to the link which is independent of presentation as long as the minimum crossing number limitation is followed Thus, links **103** and **104** may be easily assigned the $\vec{\Lambda}$ configuration, indicating isotopy with two-braid **5**

We now have a useful method for assignment of configuration to the chiral two-braid knots and links It is conjectured the oriented skew lines convention is also useful for three-braid knots and links in a presentation with the minimum crossing number The general case for knots and links is much more complex, and we do not know whether this method will allow unambiguous assignment of configuration to any chiral knot or link There is one more case, however, that must be mentioned As described above, a link with two crossings and two oriented rings is chiral In this case, the orientation of the rings is not arbitrarily assigned, but is actually a topological property of the rings Orientation of a ring is achieved if three differently colored points are embedded in the ring If the two rings, with their embedded points, are identical, then assignment of configuration to the link is straightforward

For example, consider the ring shown in Scheme 46 (**132**) It has three embedded points red, green, and blue One may arbitrarily consider the ring to be oriented red–green–blue, as shown in structure

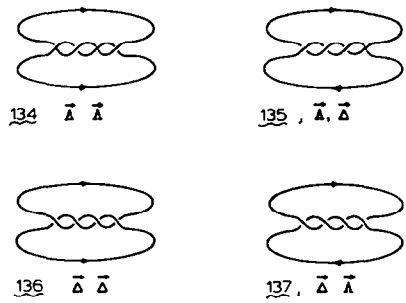



Scheme 46 The simple oriented link

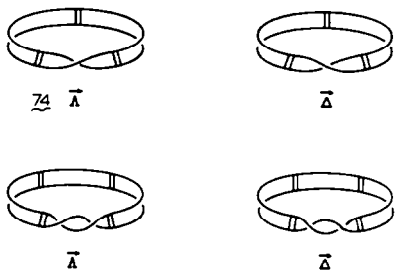
**132** This is equivalent to assigning highest priority to the red point, next highest to green, and lowest priority to blue This ring is, of course, achiral However, if two such rings are linked, then two enantiomeric configurations may result Thus, the two identical rings may be linked to give construction **4**, or its mirror image **133** In these drawings, the colored points are left off, and only the orientation of each ring is shown Application of our rules then allows assignment of the $\vec{\Delta}$ descriptor to link **4**, and $\vec{\Lambda}$ to link **133** These assignments are independent of presentation, as long as a presentation with two crossings is chosen Several presentations of the oriented links are shown in the scheme Note that our choice of priority of the points makes no difference in this assignment Thus, if we oriented the rings blue–green–red, the same configuration of the links would result That is, reversal of both arrows of a simple oriented link gives an isotopic construction Reversal of one arrow gives the mirror image

These rules for oriented links may be readily extended to molecules if the chemical basis for the orientations are the same in the two rings Thus, if the rings are DNA circles, then one may assign orientation as 5′ → 3′ If the rings are quite different, one may imagine cases where such assignment may be difficult It seems likely, however, that some variation of the CIP rules could be utilized to accomplish this orientation

When a link with more than two crossings also possesses oriented rings, then two topological elements of dissymmetry are present, and of course, topological diastereoisomerism is possible In this case, two descriptors are required to describe the configuration of the system The four possible topological stereoisomers for the two-braid link with four crossings and two oriented rings are shown in Scheme 47 The first descriptor in each case describes the configuration of the link as if neither ring were oriented The second descriptor gives the configuration of the link as if there were only two crossings With the two topological elements of dissymmetry, two pairs of enantiomeric diastereomers




Scheme 47 Topological stereoisomers of the oriented link with four crossings



Scheme 48 Configuration of the three-rung Möbius ladder enantiomers and the four-rung chiral prism enantiomers

are possible Links **134** and **136** are topological enantiomers, while **134** and **135** are topological diastereomers The topological diastereoisomerism exhibited by the links **134** and **135** is different from any of the other examples discussed so far in that *both diastereomers have the same crossing number* We conjecture that this can only occur when the isomerism derives from two or more topological elements of dissymmetry

Finally, in order to allow application of these rules to Mobius ladders and chiral prisms, another convention must be defined A standard presentation must be chosen—the wreath-like presentation as we have generally been using all along is suggested Choice of such a presentation automatically requires differentiation of the rungs and edge or edges For the Mobius ladder, assignment of an arbitrary orientation to the edge then allows simple application of the rules The Mobius ladder **74** is thus $\vec{\Lambda}$, and its mirror is $\vec{\Delta}$ as shown in Scheme 48 [69] Note that the $\vec{\Lambda}$ Möbius ladder has left-handed helical chirality For the chiral prism with two crossings, once the standard presentation is chosen, assignment of parallel orientations to the two rings making up the edges allows assignment of configuration as shown Again, the $\vec{\Lambda}$ prism has left-handed helical chirality For the Mobius ladders and prisms with higher crossing number, differentiation of the rungs and edges also easily allows assignment of configuration Note the $\vec{\Delta}$ Möbius ladder with three half twists and right-handed helical chirality affords the $\vec{\Delta}$ trefoil knot upon cleavage of the rungs

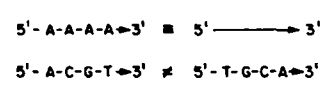
## PART VI TOPOLOGICAL STEREOCHEMISTRY OF DNA

### A *Introduction*

As pointed out in detail above, topological stereoisomerism is a rare phenomenon in the laboratory and in "small molecule" natural products chemistry In fact, topological stereoisomerism is remarkably lacking in bio-polymers as well For example, while we have made no study of the area, Professor S Benner has carefully examined the biochemical literature and found only a single protein molecule with a non-planar molecular graph [59]

There is one very striking exception to this observation—topological stereoisomerism of circular DNAs While even the DNAs are not known to exist in Kuratowski non-planar forms, the topological stereochemistry of knotted and linked DNA rings is rich and varied indeed In addition, it seems clear that this novel stereoisomerism plays a crucial role in molecular biology because the topology of DNA circles has an important influence on the conformation of the molecules, thereby controlling to some extent the host–guest chemistry of the circular DNA Thus, topological stereochemistry and Euclidean stereochemistry blend in an elegant way to dramatically influence important processes of genetics The high degree of interest generated by topological stereochemistry of DNAs among biochemists is reflected in the number of excellent review articles describing various aspects of the subject—at least six since 1978 [70]

1 *Topology of DNA strands* Before consideration of macrocyclic DNA rings, a brief discussion of the topology of acyclic DNA molecules is useful First consider single-stranded DNA As shown in Scheme 49, a single homopolymeric strand of DNA behaves topologically as an oriented line segment Thus, the tetranucleotide 5′-A-A-A-A-3′ may be modeled topologically as an oriented line The direction of the arrow is arbitrary, though biochemists generally orient the line from 5′ → 3′ as shown At the level of the sugar–phosphate backbone, ignoring the base sequence, any polynucleotide behaves as such an oriented line segment Of course, at a higher level, differentiating all four bases, the single-stranded DNA represents a much more complex topological object—an oriented line segment with embedded points that come in four "colors" Thus, the tetranucleotide 5′-A-C-G-T-3′ is topologically



Scheme 49 Topology of single DNA strands

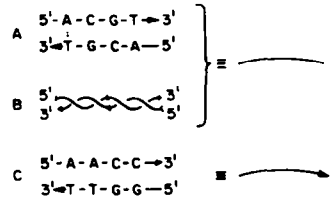
distinct from the (non-complementary) isomeric strand 5′-T-G-C-A-3′ They are, of course, constitutional isomers

Some aspects of the topology of *duplex DNAs* are illustrated in Scheme 50 By our definition, a base-paired duplex is topologically equivalent to the two separated single strands It is often useful, however, to consider the fully H-bonded duplex as a unit in which the single strands are antiparallel A fairly surprising result occurs when two identical strands are arranged in this way—the duplex is *not* oriented! Thus, when the strand 5′-A-C-G-T-3′ forms a duplex with itself, as shown in Scheme 50(A), the resulting double-stranded construction *has no orientation* This argument holds for any duplex at the level of the backbone, i e ignoring the specific sequence of bases (Scheme 50(B)) This is true even when the handedness of the helix is considered, since a right-handed double helix is right-handed from either direction In the general case, where the two strands have different base sequences, the duplex as a unit is topologically more complex, and behaves as an oriented line segment (Scheme 50(C))

2 *DNA circles* In the early 1960s the genetic material of the famous bacteriophage ΦX174 was found to exist as a cyclic single strand of DNA forming a covalent ring of about 15,000 atoms (MW = $1.7 \times 10^6$ daltons, approximately 2500 nucleotides) [71] Since then, it has become clear that cyclic single stranded and duplex DNAs are very common in nature Indeed, the chromasomal DNA of many bacteria and viruses, including the ubiquitous *E coli*, is cyclic, and cyclic DNA molecules are found in the mitochondria of most eukaryotic cells, including human cells

In 1965, four years after Wasserman's first publication describing topological stereoisomerism, Vinograd and his colleagues at Caltech reported experiments leading to a novel suggestion explaining some puzzling facts about the structure of the circular duplex DNA of the mouse polyoma virus [72] Vinograd found that the DNA exists in two different cyclic forms Using centrifugation, a covalently cyclic duplex and a linear form, in which the duplex was not cyclized, could be purified The latter material resulted from accidental cleavage of both strands of the double helix In addition, a third material, in which only one of the strands of the duplex was cleaved, could also be isolated Upon denaturation (breaking all the H-bonds of the DNA duplex), this material separated into a single-stranded circle, and a linear single-stranded chain In general, a duplex DNA with one strand broken is called a *nicked circular duplex* The puzzling problem was the following the cyclic duplex sedimented under centrifugal forces 20% faster than the singly nicked form, indicating that the two forms possess dramatically different conformations in solution While no topological stereoisomers *per se* were reported in this paper, Vinograd's ingenious explanation of his observation marks the beginning of the study of the topological stereochemistry of DNAs Soon after this work was reported, a flood of topological stereoisomers of cyclic DNA molecules were characterized in various laboratories A brief explanation of current views on the topology of circular duplex DNAs, and its controlling influence on DNA conformational properties, is presented below

3 *Linking number and linking difference* There is a tremendous weight of evidence suggesting that the most stable form of H-bonded duplex DNA in solution is very similar to the classic Watson–Crick B helix, a right-handed double helix with the DNA strands running antiparallel as shown in Scheme 50(B) Much of the strongest evidence derives from interpretation of phenomena involving topological and Euclidean stereochemistry of DNA duplex rings [73] Very recently, a single crystal X-ray analysis of H-bonded oligonucleotides nicely confirming the essentials of Watson and Crick's initial hypothesis has been reported [74]



Scheme 50 Topology of duplex DNA

For the DNA double helix in solution, recent results indicate that one complete turn of the helix occurs about every 10 5 base pairs (10 5 bp) [70f,75] This important value, 10 5 bp turn$^{-1}$, is termed the *helical pitch* Consider a linear, fully H-bonded duplex with 1050 bp Qualitatively, the preferred conformation of this molecule in solution is predicted to be a fairly rigid straight braid with 100 helical turns (1050 bp/10 5 bp turn$^{-1}$) or 200 crossings (half twists) For long duplex strands, considerable bending can occur without increasing the free energy of the system significantly Note that the crossing number of this braid is a Euclidean quantity Topologically, even considering the H-bonds as unbreakable, the crossing number of the linear "double helix" may be any integral number or zero

Now consider the product resulting upon bis-macrocyclization of the braid, in a manner exactly analogous to the Mobius strip approach to synthesis of the two-braids described above Since bonds can only form 3′ to 5′, the product will always possess an *even number of half twists* [76] The resulting *circular duplex DNA* is a two-braid link with two edges If, in the cyclization process, the ends of the braid were not allowed to twist, then our 1050 bp linear strand would give a link with a minimum of 200 crossings This crossing number is now a *topological invariant* of the construction Since this link has more than two crossings, and *also* has oriented edges, there are four possible topologically distinct stereoisomeric configurations, as described in detail in Scheme 47 for the case of four crossings To our knowledge, only one configuration has ever been considered for circular duplex DNAs—the right-handed double helix with antiparallel edges [77]

The *linking number* (*Lk*) of a circular duplex DNA is defined as one half the minimum crossing number of the two-braid link Thus, *Lk* is the number of *full twists* of a hypothetical paper strip or *ribbon* defined by the edges of the two-braid By convention, $\overrightarrow{\delta}$ crossings of the braid are given a positive sign, while $\overrightarrow{\lambda}$ crossings are negative Therefore, a right-handed helical two-braid link has a positive linking number [77] Our 1050 bp duplex circle with 200 $\overrightarrow{\delta}$ crossings has $Lk = 100$

Since the ring is very large relative to the length of a single helical turn, the H-bonding and conformational free energy of this duplex is essentially the same as that of the linear form That is, the duplex is fully H-bonded, and in its most stable conformation, with 10 5 base pairs per full turn of the helix Very little strain is introduced from bending Such a DNA ring is said to be *relaxed* The linking number of a relaxed duplex circle is denoted as *Lk*o, and in general has the value $N/10\ 5$, where $N$ is the number of base pairs When rings of this type (that is, fairly small by biological standards, but up to at least 5000 bp) are viewed in an electron microscope, they appear as relatively open circles with very few crossings This is apparently due to "spreading forces" during sample preparation If such a relaxed duplex ring is nicked (that is, one of the strands is cut) with an enzyme called *DNase*, the appearance of the ring remains unchanged The strand can be resealed with an enzyme called *ligase*, to regenerate the relaxed duplex circle

When duplex DNAs are isolated from nature, however, they generally appear in the electron microscope as quite twisted and contorted rings, with many crossings Also, such a "native" form of the ring moves faster under centrifugal forces and has a higher $R_f$ (lower retention) on agarose gel electrophoretic chromatography than a relaxed circle But, if the naturally occurring circle is nicked and then resealed, it behaves the same as a relaxed duplex circle

The fascinating explanation of this phenomenon, first proposed by Vinograd in 1965, is that naturally occurring DNA circles are *underwound* relative to the relaxed circle That is, the *linking number of the native circle is less than that of the relaxed circle* The quantity $Lk - Lko$ (or $Lk - N/10\ 5$) is called the *linking number difference*, and is always negative for native DNAs Since the linking number *Lk* is a topological invariant of the construction, and *Lk*o is simply defined by the number of bp in the circle, the linking number difference cannot change without breaking one of the strands In a circular DNA with a negative linking number difference, the helix *cannot* exist in its most stable conformation—an essentially straight rod with 10 5 bp turn$^{-1}$ Apparently, in order to minimize the conformational and base-pairing free energy, the molecule assumes a *supercoiled* conformation, with superhelical twists and coils that allow the helical pitch to remain close to 10 5 bp turn$^{-1}$, but sacrifice the "straightness" of the duplex Such a supercoiled DNA ring is more compact than the relaxed circle, explaining the observed properties of the supercoiled rings This is the essence of Vinograd's original proposal concerning the structure of the mouse polyoma virus DNA, and it is almost universally accepted today

B *Synthesis of topological diastereomers of circular duplex DNA*

That the circular duplex DNAs isolated from nature are indeed composed of two topologically linked single-stranded DNA circles is indicated by a tremendous body of evidence For example, if both

strands of the duplex are cut enzymatically, the expected linear duplex results Denaturation (breaking all the H-bonds) of such a linear duplex results in two acyclic single strands of DNA If the native circle is denatured the resulting construction still behaves as a single molecule If, however, a *nicked* duplex is denatured, a linear single strand and a separate single-stranded circle result

Biochemists have developed methods for synthesis and characterization of topologically diastereomeric DNA duplex two-braid links utilizing a variety of interesting techniques Most of these methods depend upon separation of the diastereomers by gel electrophoresis This technique can be extremely sensitive to the conformational changes occurring upon changing of the linking number of a DNA duplex Indeed, it is quite common to separate molecules differing in $Lk$ by unity

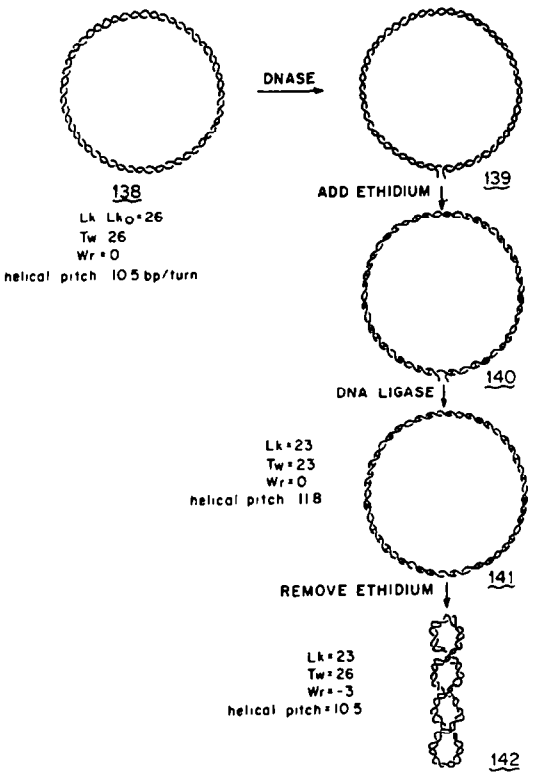
For example, if a relaxed nicked circular duplex is treated with ligase forming a covalently closed duplex circle, the product is not homogeneous by electrophoretic chromatography Rather, a family of well-resolved DNA products results, as shown in independent experiments performed in 1975 by Wang and by Vinograd [78] The best interpretation of these and many experiments performed since then agree with the proposal of those authors that the products resulting in this experiment are topological diastereomers differing in linking number, and forming a Gaussian distribution of linking numbers about the preferred value of $Lko = N/10\,5$ Thus, while the nicked duplex prefers $N/10\,5$ full helical turns, molecules with a Boltzmann distribution of Euclidean crossing numbers are present in the solution because of thermal energy available to the system Sealing the nick with ligase then locks these conformers into their topologically distinct stereoisomeric forms with $Lk - Lko = 0, \pm 1, \pm 2$, etc The maximum linking difference attainable in such an experiment depends upon the length of the DNA duplex, and upon the temperature Equations allowing estimation of the increase in free energy of the duplex with increasing linking number difference have been proposed [70 78] This increase in energy is proportional to the square of the linking number difference

If a single purified isomer is obtained off the gel and rechromatographed, it gives a single band with identical retention If this material is heated to break the H-bonds, then cooled to allow for re-formation of the H-bonded duplex, chromatography again shows a single pure topological diastereomer [73] If, however, the homogeneous topological diastereomer is nicked, then resealed, the family of diastereomers is again generated

Directed syntheses of DNA topological diastereomers have also been accomplished In one approach to preparation of DNA duplex rings with a given value of $Lk - Lko$, methods for controlling the crossing number in a nicked circle are utilized Thus, it is well known that intercalating compounds such as ethidium bromide "unwind" the helical axis by about 26° for each bound molecule If a nicked circular H-bonded duplex is treated with ethidium bromide, the Euclidean crossing number will decrease by two for every 14 bound molecules That is, the number of full helical turns is decreased by one upon intercalation of 14 molecules of ethidium bromide The binding of the intercalator, in essence, changes the helical pitch of the DNA from the normal value of 10 5 bp turn$^{-1}$ Also, the number of bound molecules may be closely controlled by the conditions of the experiment When a nicked circle thus treated is then sealed with ligase, the resulting product is a Boltzmann distribution of *relaxed circles with bound ethidium* But, the *linking number* of the major product is *less than Lko* The ethidium bromide may then be washed out of the DNA Isolation of the major product by gel electrophoresis then gives a pristine closed circular duplex with a negative linking number difference The value of $Lk - Lko$ for this product will depend upon the amount of intercalator that was bound in the nicked duplex

The approach to synthesis of DNA topological diastereomers with negative linking number differences is illustrated in Scheme 51 Thus, consider a hypothetical relaxed circular duplex **138** with 273 bp The $Lko$ for this molecule is $N/10\,5 = 26$ Appropriate treatment of this DNA with DNase could give the nicked circle **139** with 26 (now Euclidean) helical turns, or 52 crossings Treatment of this nicked DNA with ethidium bromide intercalator under conditions where about 42 molecules of ethidium bromide are bound to each DNA circle would unwind the helix, giving a relaxed nicked circle with 23 helical turns, or 46 crossings (**140**) Treatment of this material with ligase then affords the relaxed circle **141** with $Lk = 23$ This DNA has $Lk - Lko = -3$, and is a topological diastereomer of the original duplex circle **138** Removal of the bound ethidium bromide would give a supercoiled product **142** which may be purified by chromatography

A high degree of topological stereoselectivity in the synthesis of closed circular duplex DNAs is readily achievable by this strategy For example, in a recently reported series of experiments done in the laboratories of Cozzarelli, 25 diastereomers of a 1683 bp duplex circle called pAO3 were synthesized,




Scheme 51 Directed synthesis of topological diastereomers

purified, and characterized [79] These diastereomers possess $160 \pm 12, 11, 10,$ , 0 full twists, and may all be resolved on a special high-resolution composite acrylamide–agarose gel This fact is especially impressive when one considers that the covalent rings making up this link are both 10,098 atoms long

The exact number of full twists is, of course, not rigorously established for these products relative to the standards of structure elucidation currently existing in organic chemistry, and only very small amounts of material are prepared However, indirect evidence supporting the structure assignment of the molecules is very great, and it can be safely assumed that the topology of these prototypal DNA links is assigned correctly Utilizing the standard approach described in Scheme 51, Cozzarelli and co-workers synthesized and isolated topological diastereomers of the pAO3 DNA with linking number differences of $-1$ to $-12$ The product with a linking number difference of 0 (that is, the relaxed circle with 160 full twists of the two-braid) is simply prepared by ligating the relaxed nicked circle in the absence of bound ethidium, then isolating the major product When isolated in the native state, this DNA is found to have linking number differences of $-10$, $-11$, or $-12$

Methods for synthesis of the DNAs with positive linking number differences are also in common use One very elegant technique for increasing the number of crossings of a nicked circle involves binding of a protein called *gyrase* to the DNA, followed by sealing of the nick The highly novel and interesting topological stereochemistry involved in directed synthesis of DNAs with positive linking number difference by this approach is discussed in detail in Section VI F

C *Conformational analysis of circular duplex DNAs twist and writhe*

As described above, all 25 topological diastereomers of the pAO3 DNA may be resolved chromatographically by gel electrophoresis This and other evidence, including electron microscopy, strongly indicates that DNAs differing in linking number have dramatic differences in conformation in solution Interestingly, DNA with a given negative linking number difference is very similar to that with the same positive linking number difference For example, pAO3 DNA with $Lk - Lko = -4$ has a mobility on the gel more similar to the diastereomer with linking number difference of 4 than those diastereomers with linking number differences of $-3$ or $-5$ In general, the retention on gel decreases with increasing $|Lk - Lko|$ That is, the greater the absolute value of the linking number difference, the

greater the mobility on gel This, combined with other evidence, indicates that in general, the more the linking number of a DNA differs from *Lko*, the more *compact* it behaves

While the actual conformation of the DNA circles in solution is not known, some very powerful theories have been developed for consideration of the conformational picture in circular duplex DNAs These theories derive in large part from use of a Euclidean ribbon as a model for the DNA duplex Thus, a H-bonded duplex DNA may be modeled by a closed circular ribbon with an even number of right-handed half twists ($\vec{\delta}$ crossings of the edges)—i e an integer number of full twists corresponding to the linking number The midline of the ribbon is termed the *helix axis*, and *loosely* represents the central axis of the DNA duplex Similarly, the edges of the ribbon represent the DNA backbone It is the shape of the helix axis that best describes the conformational contortions responsible for the appearance of circular DNAs in the electron microscope, and for the behavior of the DNAs on gel electrophoresis and centrifugation That is, a relaxed circle has a more or less Euclidean circular helix axis, while a "supercoiled" DNA (or ribbon) has a more contorted helix axis

The conformational behavior, including the shape of the helix axis, of a mathematical ribbon is very strictly limited by the topology of the system The limitations on the shape of the helix axis may be defined in terms of a topological invariant, the linking number *Lk*, and a metric Euclidean property called the *total integrated twist*, *Tw* Treatments of this system by differential geometry were accomplished independently by mathematicians White and Fuller [80] Interestingly, Fuller's work was done in response to questions proposed to him by Vinograd relating directly to the DNA problem, while White was working on the Euclidean/topological properties of ribbons from the mathematical viewpoint independent of any thought of modelling DNA Their work has provided a very elegant model of DNA conformational behavior with great predictive value This model is a beautiful example of how topological and Euclidean geometrical properties of molecular graphs may combine to influence very important chemical properties A very short, general description of the ribbon model is presented below

A rigorous definition of *Tw* is outside the scope of this report Basically, *Tw* is a measure of how many times the edges of the ribbon turn around the helix axis—that is *the total number of turns of the helix*, measured in a frame of reference that follows the helix axis Given the intuitive meaning of "helical pitch" (length per turn), the total integrated twist *Tw* is simply the length of the helix axis divided by the helical pitch (length/length per turn = total number of turns) This equation actually serves to define "helical pitch" for a helix whose axis is twisting and writhing in space, since *Tw* is a well-defined Euclidean invariant of a ribbon Thus, helical pitch (bp turn$^{-1}$) = length (bp)/*Tw* With this definition, the author feels the intuitive meaning of "helical pitch" is preserved Note, however, that the definition of *Tw* is very precise, independent of any intuition, and *Tw* may be calculated exactly for a given ribbon conformation The value of *Tw* may be zero or any positive or negative number, not necessarily an integer The total integrated twist *Tw* for a right-handed helical ribbon is positive

It is important to note that, while the twist *Tw* seems similar to the linking number *Lk*, they are really quite different The linking number is a topological invariant, while the twist is Euclidean In fact, incredibly, the arithmetic difference between the linking number and total twist defines severe limitations on the *shape of the helix axis* If the helix axis lies on a circle, or in a plane, or indeed in any achiral shape, then $Lk - Tw = 0$ That is, the total twist *must be* identical to the linking number for such a ribbon If, however, the helix axis becomes chiral, that is if the helix axis writhes in space, or becomes supercoiled, *it is a geometrical invariant of the system that* $Lk - Tw \neq 0$

Since *Lk* is a topological invariant, and cannot change without cutting the ribbon, if the ribbon's axis is made to writhe, the twist (and helical pitch) *must* change to accommodate the deformation The difference between the linking number and total twist has been appropriately dubbed the *writhing number*, $Wr = Lk - Tw$ The writhing number is a property of the helix axis only, and may be calculated exactly for any closed curve, independent of a ribbon, let alone *Lk* and *Tw* It is a metric Euclidean quantity, and may be zero, positive or negative, not necessarily integral For a given value of *Wr*, an infinite number of shapes of the helix axis are possible But, a given value of *Wr* does *severely limit* the possible shapes of the helix axis It is this simple equation relating the shape of the helix axis to the linking number and total twist (helical pitch and length of the helix axis), which serves as such a powerful model for the conformational behavior of DNA duplex circles Indeed, the number of supercoils of a ribbon or DNA duplex is often taken simply as the writhing number—*Wr*

The relationship between linking number, linking number difference, twist and writhe is best understood by consideration of specific examples First, consider a closed circular ribbon whose length

is measured in units of base pairs. Let the helix axis of the ribbon be 1050 bp long. Also, suppose the ribbon has 100 full right-handed twists, such that $Lk = 100$, and the helical pitch of the ribbon is 10 5 bp turn$^{-1}$ Under these conditions, the total twist $Tw = 100$, $Tw = Lk$, and the helix axis may lie on a circle, or in any other achiral shape. If there were an elastic force on the ribbon tending to cause the helix axis to be straight, then the most stable shape of the axis would be a Euclidean round circle. This ribbon would correspond to a relaxed duplex DNA circle, with $Lk = Lko = 100$

Suppose $Lk$ is now changed to 99 by unwinding the ribbon (which must involve cutting the ribbon and resealing it) The axis of the ribbon can remain a circle if the helical pitch increases by one part in 100, such that $Tw = 99$ (helical pitch = 1050 bp/99 turns = 10 6 bp turn$^{-1}$) But, suppose there is a force resisting such a change in the helical pitch from the value of 10 5 bp turn$^{-1}$, as is the case in a real DNA circle What happens to the ribbon when the helical pitch is constrained to remain 10.5 bp turn$^{-1}$? In fact, the fascinating answer, rigorous for the mathematical ribbon, is that *the helix axis must become non-planar and chiral*! Note that under these conditions $Tw$ must remain 100 if the length of the helix axis does not change The chiral shape of the helix axis of such an underwound ribbon is strictly limited to one of a relatively small class of shapes defined by their writhing number—$Wr = Lk - Tw = -1$ Note also that if the helical pitch remains constant, then $Tw = Lko$ That is, in the case where the length of the helix axis and the helical pitch are constrained to remain constant at 1050 bp and 10 5 bp turn$^{-1}$, respectively, then the total twist $Tw = Lko$ Thus, if the helical pitch remains constant, $Wr = Lk - Lko$. Under these very special conditions, the number of supercoils of the helix axis, as defined by the writhing number, is equal to the *linking number difference* of the ribbon

The linking number difference gives considerable information on the shape of the helix axis once a feeling for $Wr$ is gained Scheme 52 illustrates two "limiting" conformations possible for a ribbon with $Wr = -1$ The drawings in this scheme represent the *helix axis* of a ribbon or double helix. Conformation **143** is often called a left-handed solenoidal superhelix, and **144** is called a right-handed interwound superhelix If $Lk - Lko$ is negative, it is commonly said that *negative supercoils* are induced in the helix axis (the sign of $Wr$ is negative) A left-handed solenoidal superhelical conformation such as **143** has negative supercoils, while the right-handed interwound superhelix in **144** also has negative supercoils Of course, for any linking number difference, the mathematical constraint is that $Wr = Lk - Tw$ The twist $Tw$ is only equal to $Lko$ if the helical pitch is constrained to remain constant at its value for the "relaxed" ribbon

One way to help remember the sign convention for $Wr$ is to assign descriptors by denoting the crossings as $\overrightarrow{\lambda}$ or $\overrightarrow{\delta}$. Thus, orientation of the helix axis, followed by assignment of the $\overrightarrow{\lambda}$ or $\overrightarrow{\delta}$ descriptors as described above for the case of the knots and links, gives a $\overrightarrow{\Lambda}$ (negative) conformation for both **143** and **144** Ribbons with positive $Wr$ have helical axes with $\overrightarrow{\Delta}$ conformations It is important to note that the conformation of the supercoiled ribbon is not topological, but Euclidean, and the descriptors $\overrightarrow{\Lambda}$ and $\overrightarrow{\Delta}$ here are being used to describe Euclidean shapes This technique for assigning the sign of $Wr$ assumes the conformations are drawn as shown in the scheme

It is instructive at this point to analyse the hypothetical synthesis of negatively supercoiled DNA shown in Scheme 51 in terms of $Lk$, $Tw$, $Wr$, and helical pitch. Be aware, however, that the analysis is only an approximation, even for a hypothetical duplex DNA, since the treatment is rigorous only for an infinitely thin and perfectly behaved Euclidean ribbon

The relaxed circular duplex **138** with 273 bp and helical pitch of 10.5 bp turn$^{-1}$ has $Lk = Lko = 26$ ($N$/10 5) Since the helix axis is a circle, the writhing number $Wr = 0$ (no supercoils), and $Tw = 26$ ($Wr = Lk - Tw$)

After nicking, intercalation of ethidium bromide, then resealing, the covalent duplex circle **141** is produced. This covalent duplex circle has $Lk = 23$ Thus, the linking number difference $Lk - Lko = -3$ The writhing number (number of superhelical turns), however, is still $Wr = 0$, the $Tw = 23$, and the helical pitch is now increased to 237/23 = 11 87 bp turn$^{-1}$ This DNA is a relaxed circle *with intercalated dye* The helical pitch is not the ideal value for a B helix, but is increased to a larger value by


Scheme 52 Two possible shapes for a ribbon's axis with $Wr = -1$

the intercalation Removal of the bound ethidium bromide then gives the supercoiled product **142** with the helical pitch back to 10 5 bp turn$^{-1}$ The total twist $Tw = 26$ ($Tw = Lko$ when the helical pitch is 10 5 bp turn$^{-1}$), and $Wr = Lk - Lko = -3$ (three negative supercoils)

As indicated by this analysis, supercoiled conformations may also be induced in a relaxed ribbon by changing the value of the helical pitch (i e changing $Tw$) for a given value of $Lk$ Thus, a relaxed ribbon with $Lk = Lko$ will take up a *positively supercoiled* conformation if the helical pitch is increased ($Tw$ is decreased, $Lk - Tw$ becomes positive) This is exactly the case when a relaxed covalent duplex circle is treated with ethidium bromide Indeed, such a duplex with bound ethidium apparently has positive supercoils, and is observed to behave similarly to molecules with positive linking number difference even though topologically $Lk = Lko$ For example, if the hypothetical relaxed covalent duplex **138** were treated with ethidium bromide as in Scheme 51, but *without nicking*, then the resulting material would have $Lk = 26$, $Tw = 23$ (helical pitch = 11 87 bp turn$^{-1}$ with the bound ethidium bromide), and $Wr = 3$

For a real DNA duplex, this analysis is highly oversimplified The forces acting on the ring are quite complex, of course, and the actual conformations present in solution will minimize total strain, including strain resulting from changing of the helical pitch from the ideal value, and that introduced by bending from the ideal straight rod But, all of the observations regarding DNA with a non-zero linking difference are well explained by the simple models described above That is, as the linking difference increases, the absolute value of $Wr$, and the number of superhelical turns increases, affording a more compact structure In general, it has been proposed that the interwound superhelical conformation is favored over the solenoidal superhelical conformation because the interwound conformation minimizes bending of the duplex relative to solenoidal conformations Also, if the linking difference becomes large enough, the simple model breaks down catastrophically, and severe changes in the duplex occur For example, parts of the duplex may begin to unwind, H-bonds break, and a "bubble" forms in the duplex in order to minimize strain

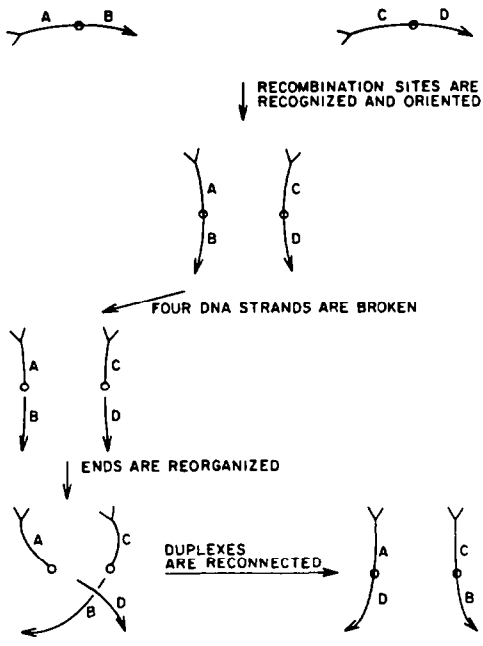
D *Naturally occurring duplex DNA catenanes*

As pointed out above, topologically linked single-stranded DNA is ubiquitous in nature All circular duplex DNAs are linked single-stranded rings with a large minimum crossing number (to our knowledge no duplex DNA circle with $Lk = 0$ has ever been characterized) But, there are other naturally occurring topologically novel constructions In 1967, Vinograd reported that about 10% of the mitochondrial DNA found in human cells grown in tissue culture exist as simple linked duplex rings with a minimum crossing number of two [81] It is now known that many cells possess catenated DNA circles At the level of the DNA backbone, such duplex catenanes are topologically achiral, since the duplex is not oriented Of course, if the single stranded circles making up the edges of each duplex have different base pair sequences, as is surely the case, the linked duplex rings exist as topological enantiomers Indeed, depending upon the linking number of each individual duplex, and upon the orientation of the two duplex rings, many topological enantiomeric and diastereomeric relationships could be present To our knowledge, the details of the topology of these duplex catenanes is not known

E *Synthesis of duplex knots and catenanes by recombination*

Genetic recombination is the important process by which a strand of duplex DNA is inserted into another strand *Site-specific recombination* involves a complex series of events As illustrated in Scheme 53, the site-specific recombination may proceed as follows (no actual mechanism is meant to be implied by this scheme) (1) recognition, pairing, and orientation of two duplex *attachment sites* AB and CD having specific base pair sequences, (2) breaking of both duplexes (four strands of DNA), (3) controlled reorganization of the broken ends, and (4) reconnection to form two new duplexes AD and CB At least two enzyme systems capable of promoting such site-specific recombination events *in vitro* under controlled conditions have recently been described [82] When the recombination sites are *both part of the same duplex circle*, the recombination event affords a constitutionally isomeric duplex product Depending upon the conformation of the circle during the recombination and upon the *relative orientation of the attachment sites*, catenated or knotted duplex DNA products will be formed Indeed, utilizing these systems, beautiful semi-directed syntheses of duplex catenanes and knots have been achieved [82a c 83]

The first synthesis of both knotted and linked duplex rings utilizing a recombination reaction was reported by Gellert in 1980 [82a] First, two isomeric 9400 bp circular duplex DNAs, called pBP86 and



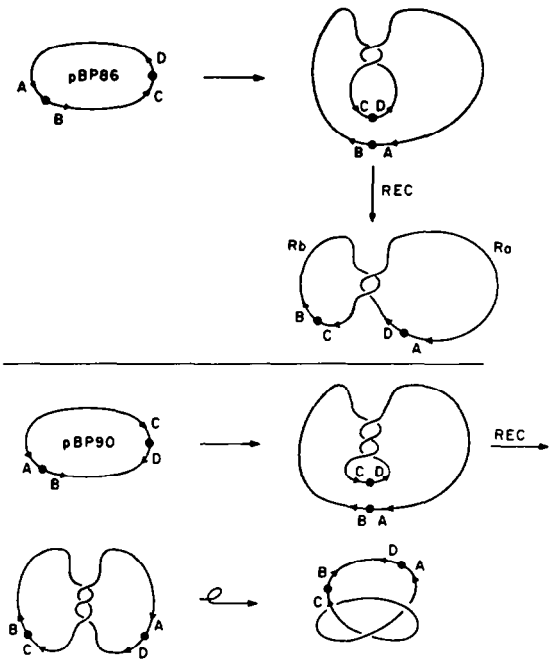

Scheme 53 Site-specific recombination

pBP90, were synthesized using standard recombinant DNA techniques Each of these circles possessed *both* required sequences for site-specific recombination (i e AB and CD in Scheme 53) The pBP86 circle has the sites running in a parallel orientation, while the pBP90 circle has the same sites, but in an antiparallel orientation In both cases, the sites are about 1500 bp apart on the DNA circle

With these two novel molecules in hand, the stage was set for a topologically stereocontrolled synthesis of duplex knots and links As illustrated in Scheme 54, an intramolecular recombination of the pBP86 circle with parallel sites *must* afford two disconnected rings, one larger ring of about 7900 bp, called Ra, and another smaller ring, of about 1500 bp, called Rb If the starting duplex is supercoiled, a two-braid catenane of Ra and Rb results Again, no actual mechanism is meant by this scenario When 25 $\mu$g (about 4 pmol) of pBP86 DNA was treated with the appropriate recombination system (purified $\lambda$ integrative recombination protein from *E coli* and a partially purified "host factor" for recombination), catenated duplex DNA products resulted The structure proof of the product DNA involves the following observations (1) The product migrated as a single band in agarose gel electrophoresis, (2) Electron microscopy of the product clearly indicates two circular products, one larger than the other The invariable presence of crossings of the product rings in the EM pictures strongly implicated topological linking of the two circular molecules, (3) When Ra was selectively cleaved utilizing an enzyme known to cleave at a specific site only present on Ra, analysis of the product by gel electrophoresis showed the presence of Rb circles, and a separate linear fragment derived from cleavage of Ra

Similar intramolecular recombination of the pBP90 circle with the same sites in an antiparallel orientation *must* give a single circular product If the starting circle is supercoiled, then a two-braid knot results, as shown in the scheme When the product of this intramolecular recombination was relaxed by nicking with DNase, a family of relaxed circles with higher mobility on gel electrophoresis than open circular forms was revealed This material showed many crossings in the electron microscope, strongly indicating the presence of knotted circles Beautiful electron micrographs of an individual molecular trefoil knot, a five-star knot, and more complex knots were obtained

Additional proof of the structures of these products was obtained by characterization of the products obtained upon treatment of the catenanes and knots with topoisomerase enzymes, as described below Note that formation of catenanes and knots by this method is operationally similar to the proposed mechanism of formation of two-braid knots and links by olefin metathesis of macrocyclic polyenes In Gellert's synthesis, however, another level of stereocontrol is achieved since the sites for recombination are organized with respect to *orientation* Thus, pBP86 gives only catenanes, while pBP90 gives only knots The actual minimum crossing number of the two-braid product of an



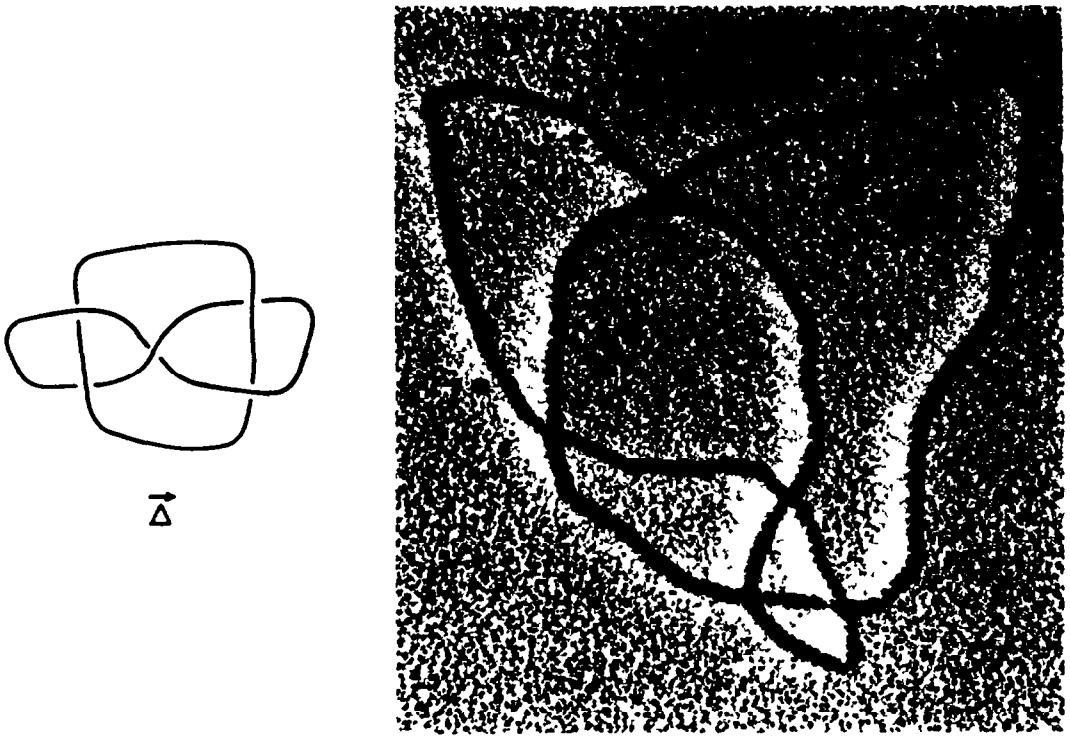

Scheme 54 Directed synthesis of duplex DNA catenanes and knots

individual recombination event, however, depends upon the conformation of the starting material at the time of recombination, and cannot be controlled completely

An especially interesting synthesis of duplex DNA catenanes has recently been reported by Cozzarelli utilizing another recombinatorial system called Tn3 resolvase [82c] In this case, treatment of the duplex DNA circle p51A, containing two directly repeated (parallel) copies of the recombination site, with pure Tn3 resolvase gave mainly simple interlocked duplex rings with a minimum crossing number of 2 However, gel electrophoretic chromatography showed the presence of a minor product (about 1% of the total catenane DNA) with a slightly higher $R_f$ on the gel than the major product It was assumed at first that this minor product was a double looped catenane with four crossings However, utilizing a new powerful method under development in their laboratory, the Cozzarelli group found that this minor catenane product had a much more novel and intriguing structure, as discussed below

Normally, it is not possible to assign the *normalization* of crossings in electron microscope images of DNAs (that is, which strand goes over, and which strand goes under) Apparently this is a result of the small diameter of the duplex chains But, there are proteins known to bind to the DNA strands, forming in essence a very thick duplex strand Indeed, when fully coated with a protein called RecA, a duplex DNA chain becomes about 100 Å thick For such a coated duplex, the normalization of crossings of DNA strands become quite visible in electron microscope pictures! When this method was applied to the minor catenane product of the above described recombination reaction, a surprising and interesting result was obtained The DNA was not a two-braid link, but rather a figure-of-eight catenane as shown in Scheme 55 The figure-of-eight catenane has a minimum of five crossings, and is topologically chiral The configuration of this link may be determined by orientation of both rings, and assignment of configuration to each crossing In this case, the initial orientation is arbitrary, and does not effect the outcome of the assignment

Direct examination of 13 molecules in the electron microscope showed that for all 13 catenanes, the *helix axes* are in the form of a $\vec{\Delta}$ figure 8, as shown (the $\vec{\Delta}$ and $\vec{\Lambda}$ figure-of-eight catenanes in this system would be topological diastereomers because of the orientation of the two rings) Thus, the recombination event most likely leads stereospecifically to formation of only one of the possible topological diastereomers of the figure-of-eight catenane, greatly limiting the possible mechanisms for Tn3 resolvase mediated recombination Indeed, we conjecture that it is topologically impossible for a single recombination event on a circular duplex DNA to afford the figure-of-eight catenane as a product The figure 8 may form from recombination of a trefoil duplex or of a figure-of-eight knotted duplex, either of which is a possible product of a single recombination of a circle The actual mode of formation of the figure-of-eight catenane in this system is not known

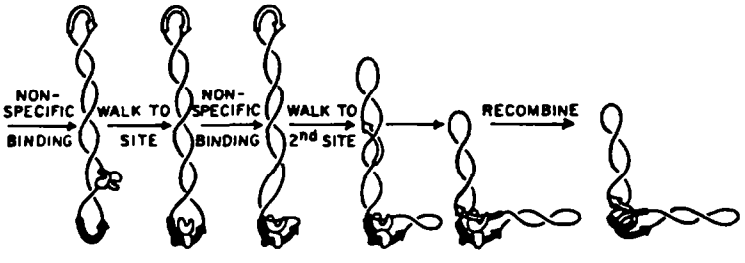


Scheme 55 The figure-of-eight catenane [83]

While the mechanism of recombination for Tn3 resolvase, or any other resolvase, is not known in detail, considerable experimental evidence suggests that the basic kind of topological transformation shown in Scheme 54 is probably not operating for Tn3 [82c] For example, Tn3 cannot recombine strands which are antiparallel, and it cannot recombine strands unless both recombination sites are on the same DNA circle A proposal explaining these results is illustrated in Scheme 56 The mechanism by which the single topological diastereomer of the figure-of-eight catenane is formed is, of course, not known From an organic chemist's perspective, one can only marvel at the power of techniques allowing assignment of the absolute topological configuration of a catenane based upon examination of 13 individual molecules

F *The topoisomerases—enzymes catalyzing the interconversion of DNA topological stereoisomers*

1 *Introduction* Several years after the discovery that native circular duplex DNA has a negative linking number difference leading to observable "supercoiling" of the ring, the first of a class of enzymes was discovered with the capability of *catalytically changing the linking number* [84] Since then, it has been shown that these enzymes can also tie knots and form links in single-stranded and double-stranded rings, and unknot or unlink the rings Wang isolated the first such enzyme, and later coined the apropos term "topoisomerase" to describe the activity of this class of proteins [70b] During the past 15 years a great deal of work has been directed at elucidation of the detailed workings of these interesting




Scheme 56 A proposed mechanism for Tn3 resolvase [82c]

catalysts Topological stereochemistry has played and continues to play a key role in limiting mechanistic possibilities, similar to the role of Euclidean stereochemistry in the study of organic reaction mechanisms A short discussion of the chemistry and topological stereochemistry of the topoisomerases therefore seems a fitting conclusion for this report

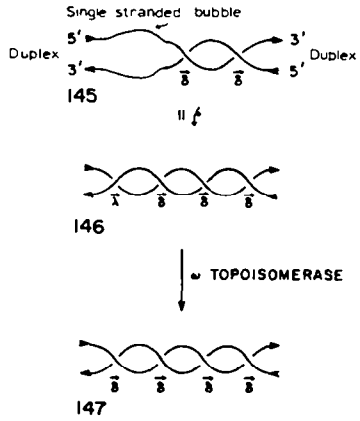
The topoisomerases fall into two main categories *Type 1* topoisomerases form a *transient single-stranded break*, allow a DNA chain to pass through the break, then reseal the strand, all in one catalytic operation *Type 2* topoisomerases form a *transient double-stranded break*, allow a DNA chain to pass through, then reseal the duplex without allowing rotation of the broken ends relative to each other

2 *A prototypal type* 1 *topoisomerase from* E coli The first topoisomerase to be isolated was initially recognized as a factor capable of relaxing negatively supercoiled DNA [84] This protein, isolated from *E coli*, and called $\omega$, is typical of a class of type 1 topoisomerases isolated from a variety of prokaryotic sources The *E coli* enzyme is the most extensively studied topoisomerase, and possesses the following basic activities (1) relaxation of negatively supercoiled duplex circles, (2) formation of knotted rings from single-stranded circles, and removal of the knots, (3) formation of a relaxed circular duplex from complementary single-stranded rings (i e formation of catenanes from single-stranded rings), and (4) formation of catenanes and knots from singly nicked duplex circles All of these transformations only proceed under conditions where there is a negative free energy change, and no high energy cofactor is required

In the cell, the type 1 topoisomerases are thought to help control the topology of duplex DNA by relaxing duplexes with a large negative linking number difference The relaxing reaction exhibits considerable *topological stereoselectivity* A duplex circle with a high negative linking number difference is rapidly relaxed As *Lk* approaches *Lko*, however, the reaction becomes much slower Duplex DNA circles with a positive linking number difference are unchanged by the enzyme Thus, *E coli* $\omega$ effectively only *increases Lk*, and only for a duplex with a negative linking number difference

A typical reaction catalyzed by $\omega$ would proceed as follows Consider our hypothetical 1050 bp duplex circle, with $Lk = 95$ ($Lko = 100$) Treatment of this DNA with $\omega$ would produce a family of DNAs with $Lk = 96, 97, 98$, etc After a long period of time, a Boltzmann distribution of DNAs with the major diastereomer having $Lk = 100$, would be generated This product distribution is exactly that which would be obtained upon treatment of a relaxed nicked circle with ligase The topological transformation occurring in this process is illustrated in Scheme 57 Consider a section of the DNA circle described above, with a single-stranded "bubble" in the duplex as shown in structure **145** Topologically, this construction is equivalent to **146**, where an added $\vec{\delta}$ and $\vec{\lambda}$ crossing are shown The $\omega$ enzyme changes the topology of **146**, catalyzing the conversion of a $\vec{\lambda}$ crossing into a $\vec{\delta}$ crossing to afford product **147** This topological diastereoisomerization increases the minimum crossing number of the product by two, increasing *Lk* by one

In addition to the relaxation reaction, $\omega$ also catalyzes the knotting and unknotting of single-stranded DNA rings [85] When a single-stranded circular DNA called fd is treated with $\omega$ under conditions known to promote formation of intramolecular H-bonded helical regions, knotted single-stranded rings are produced The knotted rings are not homogeneous, and are quite complex, with many crossings Similarly, when the single-stranded knotted rings are treated with $\omega$ under conditions

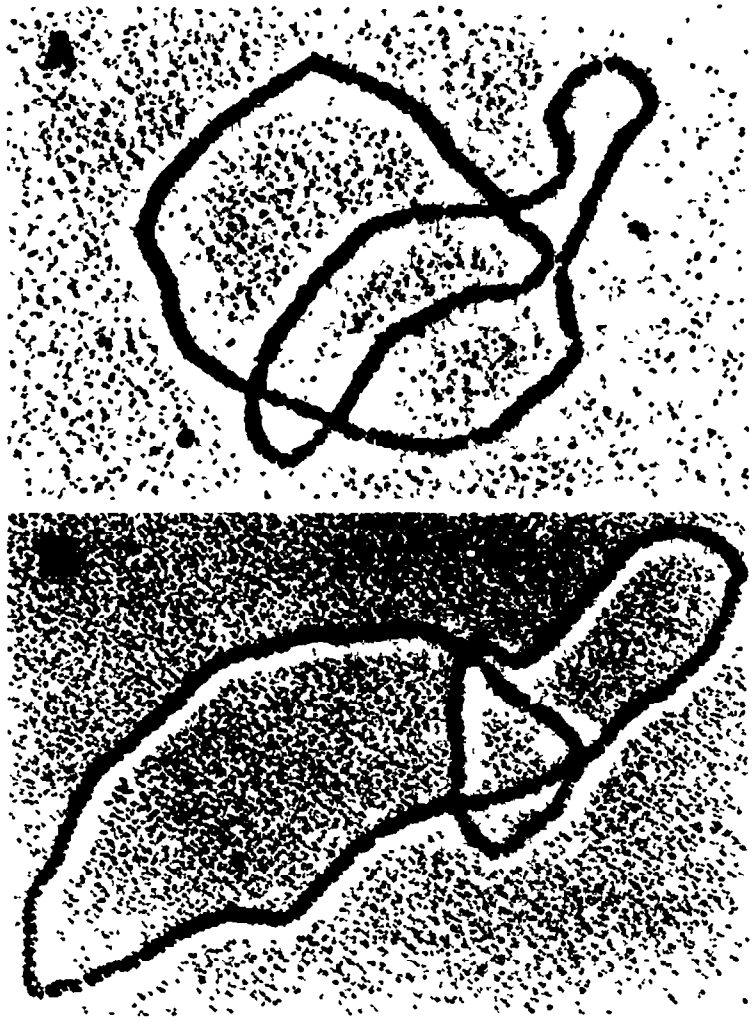



Scheme 57

known to inhibit formation of H-bonds, the rings are converted back to unknotted circles The topology of these products is indicated by their appearance in the electron microscope under identical sample preparation conditions, the behavior of the products on centrifugation and gel electrophoresis (knotted rings have a higher mobility, and smaller hydrodynamic radius), and also by the fact that all of the products are converted to identical single-stranded linear molecules upon enzymatic introduction of one single-strand break

In a process functionally similar to the knotting reaction, $\omega$ is able to link single-stranded circles When a mixture of complementary single-stranded circles (that is, two DNA circles whose base sequences are complementary) of PM2 DNA (about 10,000 nucleotides) are treated with $\omega$ under conditions promoting the formation of H-bonds, then the protein catalyzes the formation of a *relaxed duplex* ring This process involves first linking of the two complementary single-stranded rings ($Lk = 0$) to form a single-stranded catenane with $Lk = 1$ Such a link has a very large negative linking number difference (about $-950$), and is rapidly converted to a duplex circle with a smaller linking number difference Eventually, a fully relaxed circle results, after about 950 catalytic cycles of the enzyme

Finally, another activity of $\omega$ has recently been reported When a *nicked duplex* is treated with the enzyme, knotted and linked nicked duplex products can result [86] For example, when 40 $\mu$g of pRR51 (5750 bp) circular duplex DNA is singly nicked by treatment with DNase, then allowed to react with $\omega$, trefoil knotted nicked circles can be isolated by gel electrophoresis in 10% yield [83] Topologically, such a molecule consists of a single-stranded covalent trefoil knot, and a separate linear strand The proof of structure of the knotted product and determination of the topological stereoselectivity of the knotting reaction were accomplished utilizing electron microscopy coupled with the strand-fattening procedure



Scheme 58 RecA coated trefoil knots [83]

described above Thus, when 25 $\mu$g (about 6 5 pmol) of the purified trefoil knotted nicked duplex circles were treated with *E coli* RecA protein, then examined by electron microscopy, both topological enantiomers of the trefoils were observed, as shown in the beautiful electron micrographs in Scheme 58 Forty individual molecules were examined by a panel of observers, where the orientation of the crossings were established by examination of each individual crossing with the others masked Of these knots, 18 were $\vec{\Delta}$, and 22 were $\vec{\Lambda}$, showing that indeed trefoils are produced, and that no topological stereoselectivity is evidenced in the process by which the crossings are introduced

The results described above, along with considerable other evidence, strongly implies the following mechanism for $\omega$ The protein first binds a single-stranded region of DNA There is very little site specificity in this binding process A single-strand break is then introduced into the DNA, with formation of a covalent bond between a phenolic hydroxyl group of a tyrosine moiety of the enzyme, and the 5′ phosphate of a nucleotide A strand of DNA is then passed through the break, which is then resealed At no time during the catalytic process does the enzyme "let go" of the ends of the broken DNA strand The DNA strand that passed through the break can be single stranded or duplex, and has no base sequence requirement

As indicated by the determination of the configuration of the nicked duplex trefoils generated by $\omega$, no stereoselectivity is evidenced in this particular topological isomerization Indeed, given the mechanism described above, this fact is not surprising Consider the local structure at the active site of the enzyme during this knotting reaction A duplex is passed through a broken single strand The single strand is oriented But, since no base-pair selectivity is exhibited by the enzyme, the duplex behaves as a non-oriented line Thus, *locally*, ignoring the base sequence, the transition state is not topologically chiral! The enzyme *cannot* differentiate the *sign* of the change in crossings that results in knot formation without other information

The process of passing a single-stranded DNA through such a break is, however, fundamentally different In this case, both the breaking strand and the passing strand are oriented, and the transition state is topologically chiral The enzyme *could*, in principle, differentiate the two processes, and exhibit a mechanism-based topological stereospecificity, even ignoring any base sequence Indeed, $\omega$ can only *increase* linking number of negatively supercoiled substrate This could be due to the availability of single-stranded segments of highly negatively supercoiled DNA allowing the process to occur Since relaxed, or positively supercoiled DNA has no single-stranded region, the observed stereoselectivity could be due to the inability of the enzyme to react at all with positively supercoiled substrates It is certainly intriguing, however, to consider the possibility that $\omega$ is intrinsically topologically stereoselective

A judgement concerning this question could be made if the configuration of the first single-stranded c[illegible]anes formed when $\omega$ links two complementary single-stranded circles were determined As q[illegible]ribed above, the product derives from the link with $Lk = 1$ However, it is possible that links with l[illegible] $= -1$ are also produced, but do not lead to final product formation, and are simply unlinked, then c[illegible]verted to the product If only links with $Lk = 1$ are actually produced, the enzyme would most likely be intrinsically topologically stereoselective If, however, both links with $Lk = 1$ and $Lk = -1$ are produced, then a topologically stereorandom process is indicated No determination of this kind has yet been accomplished to our knowledge

3 *Type 1 topoisomerases from eukaryotes* Several years after the isolation of $\omega$, a similar activity was observed in mouse cells The type 1 topoisomerases isolated form eukaryotic cells also change the linking number of DNA duplex circles by one, but they differ in several topologically interesting respects from the $\omega$ protein First, eukaryotic type 1 topoisomerases are evidently topologically stereorandom, since they are able to relax both positively and negatively supercoiled circles Also, when interrupted during the reaction, the DNA is found covalently bound to the 3′ phosphate via a tyrosine residue of the protein

4 *Type 2 topoisomerases—DNA gyrase* In 1976, Gellert first reported isolation of a new enzyme from *E coli* called gyrase, capable of changing the linking number of supercoiled DNA circles [87] *E coli* gyrase changes the linking number in units of two, and is now recognized as prototypal of a whole class of enzymes called type 2 topoisomerases Members of the class of *prokaryotic* type 2 enzymes similar to *E coli* gyrase perform the following interesting operations (1) the gyrases are the only enzymes known capable of *introducing* negative supercoils into duplex DNA circles in an endergonic process requiring the presence of ATP, (2) these enzymes can relax negative supercoiled DNA in the absence of ATP, (3) gyrase can link and unlink, or knot and unknot covalent duplex DNA circles, and (4) in the absence of

ATP, bound duplex circles, upon treatment with $\omega$ or by nicking then closing, attain a positive linking number difference

In the presence of ATP, DNA gyrase catalytically *decreases* the linking number of a relaxed duplex circle generating a product with a negative linking number difference The maximum *linking number difference density* [$(Lk - Lko)/Lko$] attainable at high enzyme concentration is about $-0.01$ Thus, if our 1050 bp duplex, as a relaxed covalent circle with $Lk = 100$, were treated with gyrase and ATP for a long period of time, a family of supercoiled DNAs with $Lk$ of about 90 would be formed in an endergonic process driven by ATP hydrolysis In addition, if a homogeneous DNA circle is used as starting material, *only products where the linking number is changed in units of two* are produced [88] If a pure diastereomer of the 1050 bp circle with $Lk = 100$ were treated with gyrase in the presence of ATP, products with $Lk = 98, 96, 94$, etc down to about $Lk = 90$, would be formed It is easily demonstrated by experiments involving gel electrophoresis that none of the products corresponding to a unit change in $Lk$ are produced in this reaction The gyrase enzyme thus changes the topological minimum crossing number of the duplex link by *four*

In the absence of ATP, gyrase can relax duplex DNA circles with a negative linking number difference This relaxing reaction is much slower than the ATP driven supercoiling process, and shows topological stereoselectivity That is, in the absence of ATP the linking number can only be increased, and positive supercoiled DNA is unchanged

Some of the strongest evidence regarding the basic mechanism of gyrase action derives from topological isomerizations of covalent duplex circles catalyzed by the enzyme For example, when treated with gyrase in the presence of ATP, the DNA catenane shown in Scheme 54 became unlinked Electron microscopy showed the presence of the intact smaller Rb circles and larger Ra circles in the product No single-stranded material was produced Gyrase was also able to catalyze the topological diastereoisomerization of knotted DNA to the open circular form In addition, gyrase has the ability, under appropriate conditions and in the presence of ATP, to tie knots in unknotted circular duplexes, and to link circles to form duplex catenanes

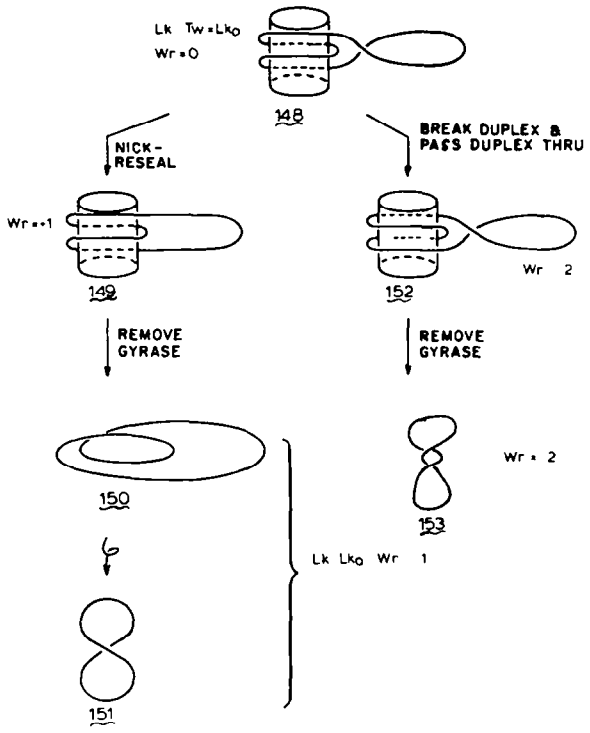
Finally, more subtle information regarding the mechanism of action of gyrase has been gleaned from studies on the binding of the protein to duplex DNA [89] When gyrase binds to a duplex DNA in the absence of ATP, about 160 bp are protected from reaction with DNA cleaving enzymes, indicating that the gyrase molecule binds to a rather long stretch of the DNA chain Also, if nicked circular duplex DNA is first bound to gyrase, then treated with ligase, then purified away from all protein, the product DNA circle has a *positive linking number difference*! The same result is obtained if a covalently closed duplex is treated with gyrase, then the complex is treated with $\omega$ or a eukaryotic type 1 topoisomerase This property of gyrase was utilized in the directed synthesis of positively supercoiled pAO3 DNA circles described above

All of these observations, and many others, indicate a mechanism of gyrase activity involving formation of a double-stranded break in a DNA duplex, passage of a duplex strand through the break (sometimes called sign inversion), then resealing the break without ever "letting go" of the two broken ends or allowing them to twist with respect to each other The fact that in the presence of ATP the linking number is always *decreased* shows that gyrase is topologically stereoselective Indeed, this topological stereoselectivity is thought to be intrinsic to the mechanism of action of the enzyme A possible mechanism, in very rough outline, is presented in Scheme 59

First, in an ATP independent step, a relaxed DNA duplex ($Lk = Lko$) wraps around the enzyme, indicated as a cylinder in the scheme, in a *stereospecific manner*, to afford a duplex with a *right-handed* (positive) solenoidal turn In order to maintain the preferred helical pitch, supercoiling must occur elsewhere in the duplex In structure **148**, a *right-handed* (negative) interwound superhelical turn is shown Thus, for the duplex complex **148** $Lk = Lko = Tw$, and $Wr = 0$ The region where the DNA is close to the enzyme is about 160 bp (about 550 Å) long

If this bound DNA is now nicked, the interwound superhelical coil can relax to minimize bending, *adding an additional Euclidean turn to the helix*, while the solenoidal coil remains since it is stabilized by binding to the protein Resealing of the relaxed complex then affords product **149**, wherein the duplex circle has $Lk - Lko = Wr = 1$ The same product may be obtained by treatment of complex **148** with $\omega$, which accomplishes the nicking and resealing in a single step Removal of the protein then gives a free positively supercoiled DNA circle (**150** or **151**) with $Wr = 1$ This method was utilized to prepare the positively supercoiled pAO3 DNAs described above

In the presence of ATP, complex **148** undergoes a different topological transformation, as shown




Scheme 59 Possible mechanism for gyrase

The duplex is broken and a duplex strand is passed through the break in a stereospecific manner to afford complex **152**, with a *left-handed* (negative) solenoidal coil This process reduces the linking number by two Thus, for complex **152**, $Lk - Lko = Wr = -2$ Note that complex **152** has a negative solenoidal coil and a negative interwound superhelical coil The total twist, however, is still equal to *Lko*—that is, the helical pitch has remained at the preferred value of 10 5 bp turn$^{-1}$ Removal of the protein now affords a free negatively supercoiled duplex product with $Lk - Lko = Wr = -2$ (**153**) An interwound superhelically coiled conformation for this product is shown in drawing **153** Of course, the scenario described in Scheme 59 is highly speculative Other possible mechanisms are described in the literature [70e 82a,88 89b,90] The mechanism shown here is simply meant to illustrate one possible way in which gyrase may operate A definitive answer to the mechanistic questions posed by these enzymes is yet to come

Other type 2 topoisomerases have been isolated from both prokaryotes and eukaryotes All change the linking number of circular duplex DNAs in units of two, can knot and unknot, or link and unlink closed duplex circles, and all require ATP [90] Only the prokaryotic type 2 topoisomerases exemplified by *E coli* gyrase, however, can drive the formation of negatively supercoiled DNAs

## CONCLUSION AND POSTSCRIPT

In this report I have attempted to impart a feeling for the novelty and esthetic appeal of topological stereochemistry While a few synthetic organic chemists are just now preparing molecules exhibiting topological stereoisomerism, an army of biochemists is working to elucidate biologically important aspects of topological stereochemistry in nature The story is still unfolding and will certainly provide challenging and interesting problems for practitioners in the future Indeed, since completion of the manuscript of this report in the Fall of 1983, additional syntheses in the field of small molecule topological stereochemistry have been described Very brief mention of this work is given here as a postscript The reader is referred to the original literature for details

Schill has completed a total synthesis of the first catenane in which both rings are simple polymethylene chains [91] The synthesis involves first formation of a functionalized rotaxane, then macrocyclization and removal of all functionality In this catenane, one ring has 28 members and the other has 46

Sauvage has reported a very elegant and efficient synthesis of a highly functionalized catenane by utilizing a Cu(I) cation as template [92] The linked rings of the product are composed of polyethyleneoxy chains closed by 2,9-diphenyl-1,10-phenanthroline moieties Novel complexation properties of this highly interesting ligand system derive from a nice interplay between the topology and topography of the system

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[8] This definition of topological enantiomers differs in an important way from the accepted definition of Euclidean enantiomers That is, in conventional stereochemistry enantiomers must be rigid mirror images Topological enantiomers, however, need not be rigid mirror images The trefoil knot **2** is but one of an infinite number of presentations of knots isotopic with **2** Any of these presentations is a topological enantiomer of **3**

[9] To our knowledge, there are no one-dimensional objects known to be topologically chiral which do not possess one of the elements of topological dissymmetry given in the first paragraph of Part I D The conjecture that one of these elements is necessary for topological chirality, however, is quite weak at this point

In collaboration with the author, Professor Jonathan Simon, of the Department of Mathematics at the University of Iowa, has explored this conjecture Based upon considerable mathematical prior art it seems likely that there exist chiral embeddings of unmarked planar graphs containing no chiral knots or links A search for specific examples is under way More generally, the question of whether it is theoretically possible to specify a complete list of elements of topological dissymmetry is unresolved

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The definition of the sign of *Lk* is ambiguous for the special case of $Lk = 1$, since in this case *helical* $\vec{\delta}$ or $\vec{\lambda}$ crossings cannot be defined The $\vec{\Lambda}$ link with two crossings is assigned $Lk = 1$ The duplex DNA described in the previous paragraph thus has

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