Pergamon

0960-0760(95)00107-7

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1–6, pp. 603–613, 1995

0960-0760/95 $9.50 + 0.00

Chemistry and Conformation of Vitamin D Molecules

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1α,25-Dihydroxyvitamin D_3 (1,25) is a structurally unique steroid hormone because it not only possesses the complete 25-hydroxycholesterol side chain, but most notably, it possesses a seco-B triene structure (it lacks a B-ring and is usually depicted in a non-steroidal, extended conformation). In contrast, the classical steroid hormones possess a truncated side chain (progesterone, cortisol, and aldosterone) or no side chain (estradiol and testosterone) and they all possess the fully intact ABCD steroid rings. These structural differences render the seco-B-steroid 1,25 considerably more conformationally flexible. Since 1,25 is now known to target a myriad of tissues where specific interactions occur to produce an array of biological responses, it is of interest to determine whether different topologies of 1,25 (resulting from different conformational orientations of 1,25) are necessary to interact effectively at the different target sites. The array of biological responses include both non-genomic and genomic effects and there is considerable promise for the efficacy of 1,25 analogs as chemotherapeutic agents in a variety of human disease states. For the non-genomic calcium transport response of transcaltachia, the finding that two 6-s-*cis* locked analogs, 1α,25-dihydroxyprevitamin D_3 (pre-1,25) and 1α,25-dihydroxylumisterol$_3$ (1,25-Lumi), are equipotent to 1,25, points strongly to the involvement of the 6-s-*cis* conformer of 1,25 as the biologically active conformer. Since there is a continuum of easily interconvertible 6,7-single bond conformers of the seco-B ring available to 1,25, conformational minima (either local or global) may have little to do with the manner in which 1,25 is bound to receptor. For the genomic calcium transport response, and for other genomic (or non-genomic) effects, there is no clear evidence whether the steroidal (s-*cis*) or non-steroidal (s-*trans*) conformer of 1,25 is involved. In order to address this matter further, efforts are underway to evaluate other conformationally locked analogs of 1,25 which might mimic either the planar 6-s-*trans*-1,25 or some intermediate conformer between it and the planar-6-s-*cis* form.

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INTRODUCTION

It is well established that vitamin D_3 (D_3) is successively hydroxylated in the liver and then the kidney to produce the seco-B steroid, 1α,25-dihydroxyvitamin D_3 (1,25). The latter is considered to be the active form of the vitamin D hormonal system and it is this substance which migrates to a myriad of target tissues where specific interactions occur to produce an array of biological responses (Fig. 1) [1].

At least ten research areas can be identified as current frontiers of international efforts in the vitamin D field (Fig. 2) [2, 3]. Major efforts are under way to develop these areas, both at the basic and applied levels including the development of new drugs for clinical applications. This paper will focus principally on one of these research areas, namely on 1,25 and its analogs as developed more fully in Fig. 3. One of the major goals concerns the development of an understanding of the topology or 3-dimensional shape of the hormone 1,25 and ultimately the hormone-receptor complex [4]. This entails a need for exhaustive structural studies of 1,25 and its analogs and, of course, the coupling of

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24–29 September 1994.
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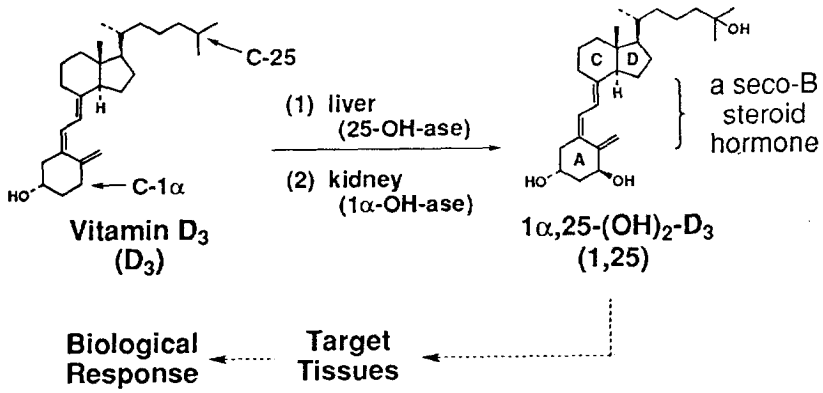


Fig. 1. Metabolism of vitamin D_3 (D_3) to 1α,25-dihydroxyvitamin D_3 (1,25).

this information with biological data for developing structure–function analyses. Achieving these goals demands an interdisciplinary approach which is expected to lead to a basic molecular understanding of the mechanism of action of vitamin D and the development of analogs with selective physiological action. Many laboratories have been especially heavily engaged in developing analogs that elicit high cellular differentiation and low cellular proliferation while exerting minimal toxic hypercalcemia.

The lynch-pin to achieving these goals requires chemical synthesis developments, but this topic in terms of the Riverside effort is only briefly summarized in Fig. 4. These include the Lythgoe-Mouriño dienyne approach 1 [5], our vinylallene approach 2 [6], the Lythgoe-Roche phosphine oxide approach 3 [7], and more recently the Trost seco-A ring enyne approach 4 [8]. A number of other routes are also available. It is probably safe to say that given enough time and resources, virtually any analog or metabolite, with and without isotopic label can be obtained by synthetic organic chemists. This optimism is, however, tempered by the fact that some analogs require many months to prepare and there is no universal chemical synthesis method.

The main topic of this paper, however, will focus on the structure of 1,25 and its ability to dynamically change its shape through conformational (by single bond rotation) [4] or chemical isomerism (by a [1,7]-sigmatropic hydrogen shift, [1,7]-H shift) [9]. Studies directed towards forcing a shape on to the 1,25 ligand through conformational or configurational locking will

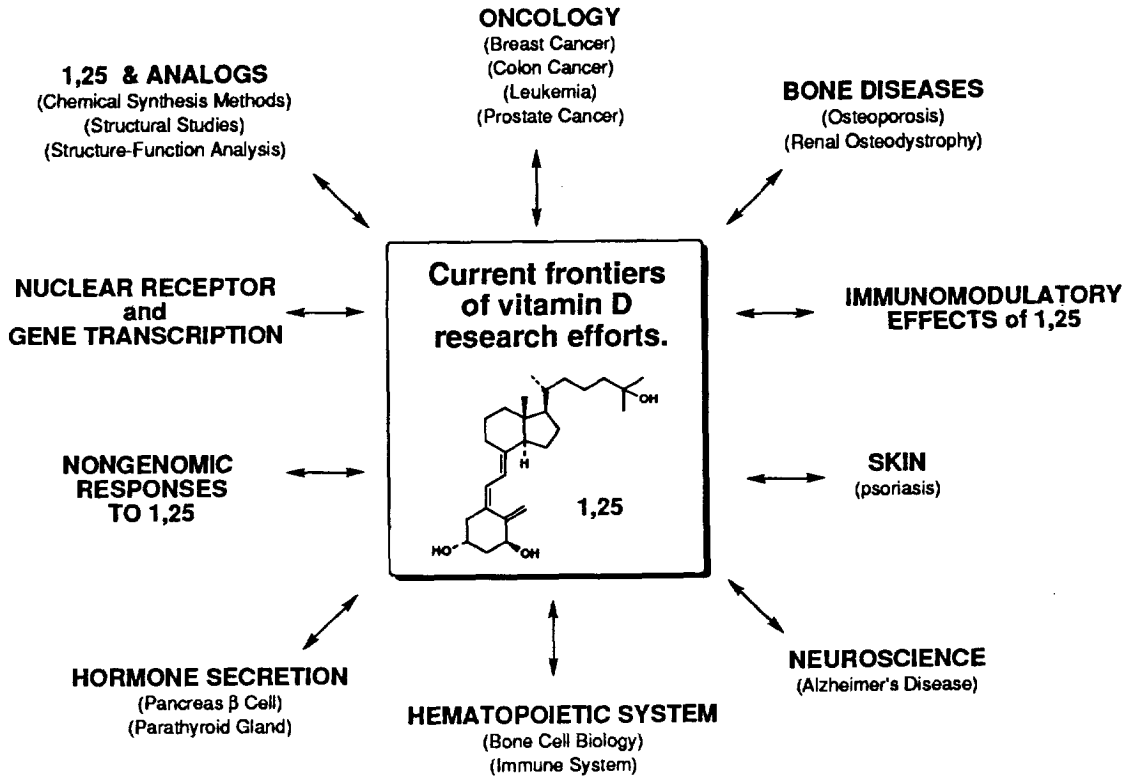


Fig. 2. Current frontiers of vitamin D research efforts.

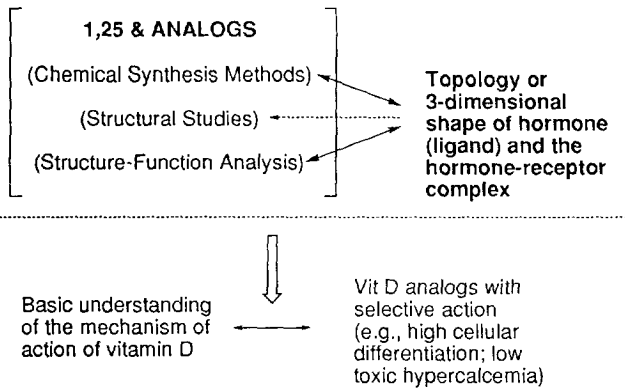


Fig. 3. Chemical, structural and structure–function studies of 1,25 and its analogs.

be discussed. In considering any structure–function analysis of a protein ligand such as 1,25, the entire molecule must ultimately be evaluated. However, it is convenient to subdivide the structurally dynamic steroid 1,25 into four distinct "geographic regions" (Fig. 5): the "southerly" A-Ring, which can exist as an equilibrating pair of chair conformers; the seco-B-ring triene, which may exist in the limiting 6-s-*trans* and less stable 6-s-*cis* forms by 6,7-single bond rotation; the relatively rigid CD-ring, a *trans*-hydrindane unit which in our analysis is assumed to be an anchor; and finally, the "northernmost" side chain, which is virtually free to rotate about six single bonds.

In addition to the dynamic behavior of 1,25 resulting strictly from very rapid single bond rotations, there must be included a relatively slow chemical reaction that transforms 1,25 into 1α,25-$(OH)_2$-previtamin D_3 (pre-1,25). These are collectively detailed in Fig. 6. The upper panel highlights the rapid chair–chair A-ring inversion process wherein C5 (steroid numbering) is tethered via the intercyclic diene unit (C5=C6—C7=C8 in its 6-s-*trans* conformation) to C8 of the relatively rigid CD ring anchor. The middle panel depicts the 6-s-*trans* and 6-s-*cis* limiting conformations of the seco-B ring triene as well as the slow chemical process, which transforms 1,25 to pre-1,25. Finally, the bottom panel depicts by means of curved arrows, the six single bonds about which the side chain may rotate [4, 10].

The highly dynamic behavior of 1,25 (Figs 5 and 6) raises interesting challenges and questions. How does the ligand (1,25) interact with the myriad of target tissue receptors and is the ligand in fact identically bound to the receptors at the different target sites? In addition, just how does the ligand–receptor complex induce the remarkable array of physiological effects alluded to in Fig. 2? An intriguing scenario is that the stereostructural demands of the 1,25 receptors at the various target sites differ; that is, although 1,25 may be the natural ligand for each, different topologies of 1,25 bind to the different receptors. If the ligand binding site of the various receptors differ, this provides an

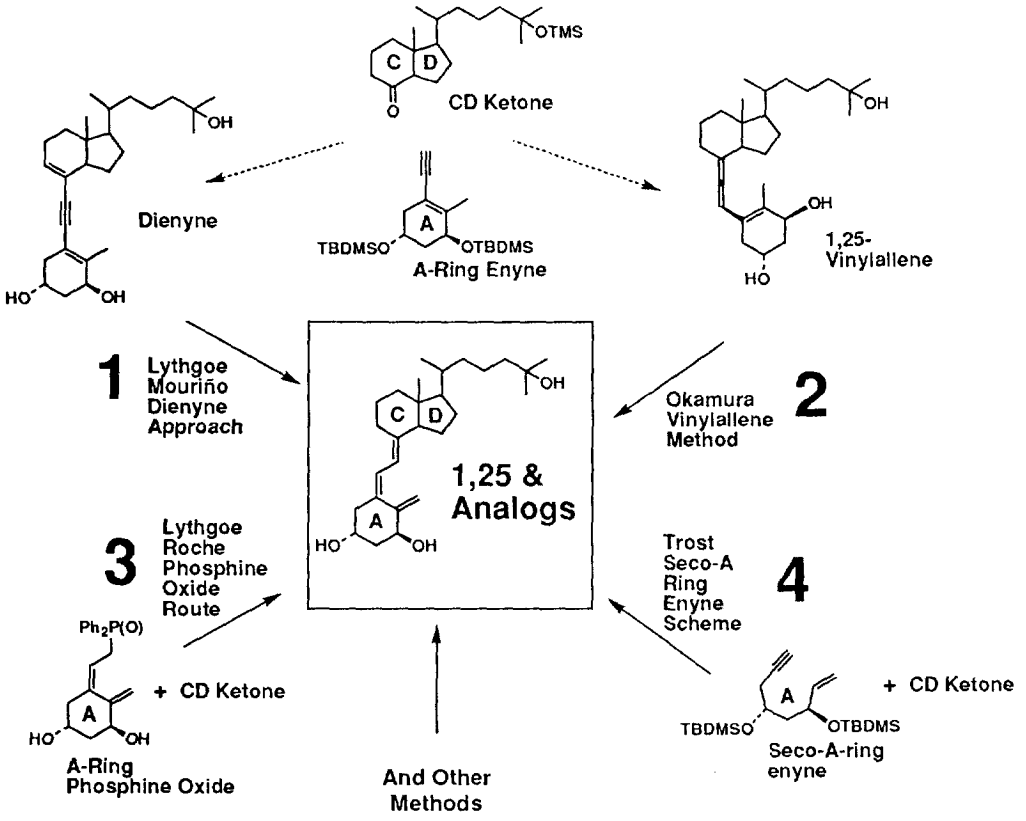


Fig. 4. Chemical synthesis methods of 1,25 and its analogs used frequently in Riverside.

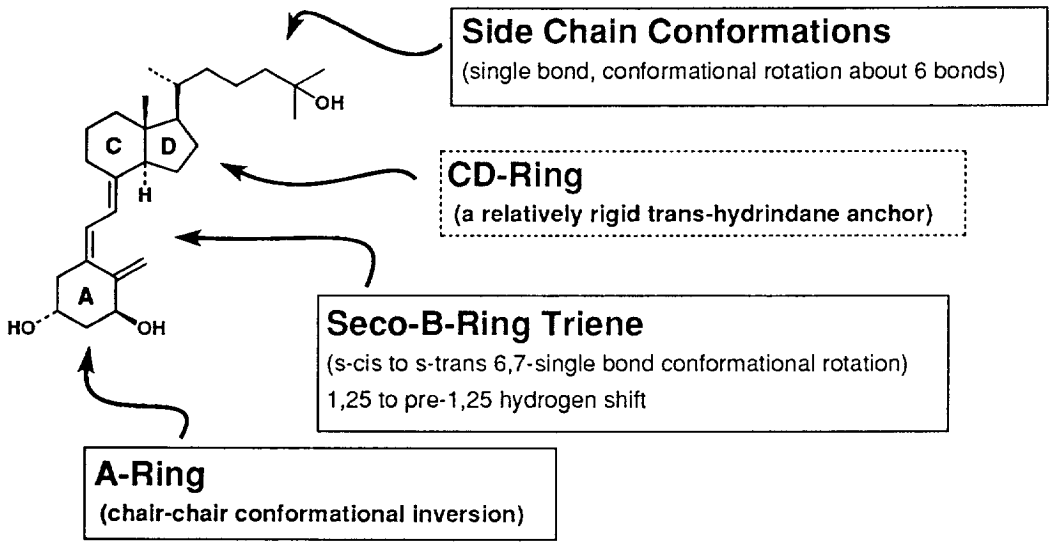


Fig. 5. The dynamic A-ring, seco-B triene, CD ring and side chain of the seco-steroid 1,25.

opportunity to design analogs of 1,25 with different shapes to exert selective biological action.

PRE-1,25

The hypothesis just mentioned, certainly a testable one, emerged serendipitously from an effort to determine whether pre-1,25 possesses its own unique biological function *in vivo* [11]. It is known that 1,25 in solution slowly equilibrates with small amounts of pre-125; thus, although there is no direct evidence, the supposition for purely chemical equilibrium reasons is that pre-1,25 must exist *in vivo*. In order to examine whether this equilibrium isomer of 1,25 possesses any biological activity in its own right, pre-1,25 and an appropriately deuteriated form of pre-1,25 [namely, 9,14,19,19,19-pentadeuterio-pre-1,25 (pre-1,25-d_5)] were chemically synthesized and subjected to kinetic studies for their rearrangement to 1,25 and 9,9,14,19,19-pentadeuterio-1,25 (1,25-d_5) (Fig. 7), respectively [9]. The kinetic investigations at 37°C established that pre-1,25 and pre-1,25-d_5 rearranged to 1,25 and 1,25-d_5 with half-lives of 13.4 and 81 h, respectively. While the presence of deuterium exerts but a small effect on biological activity (1,25 vs 1,25-d_5 and pre-1,25 vs pre-1,25-d_5), the presence of deu-

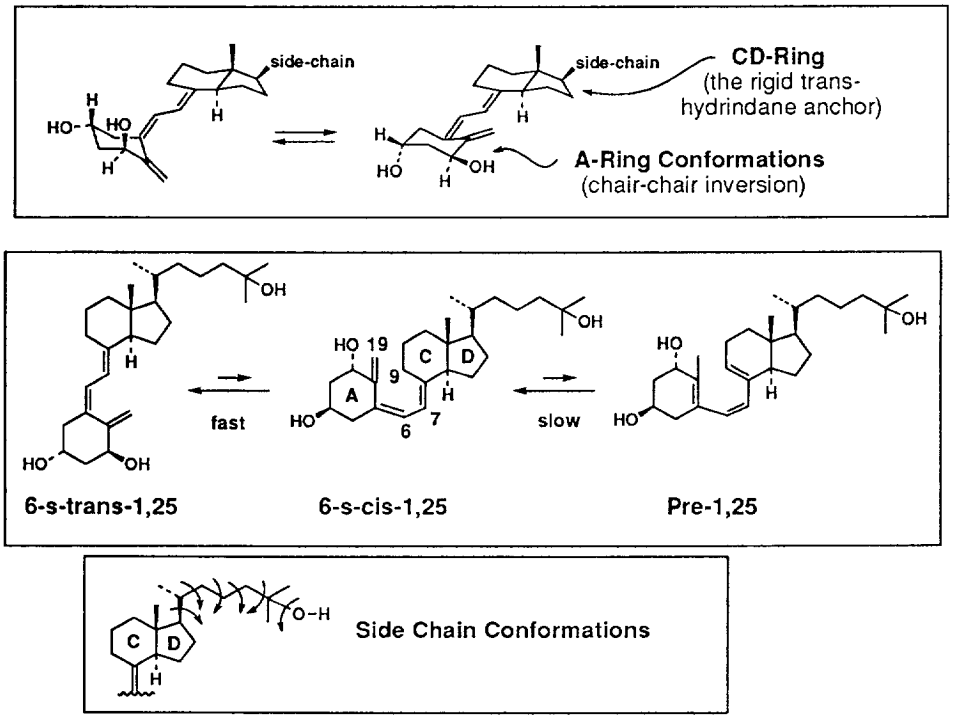


Fig. 6. Detailed description of the structurally dynamic seco-B-steroid 1,25.

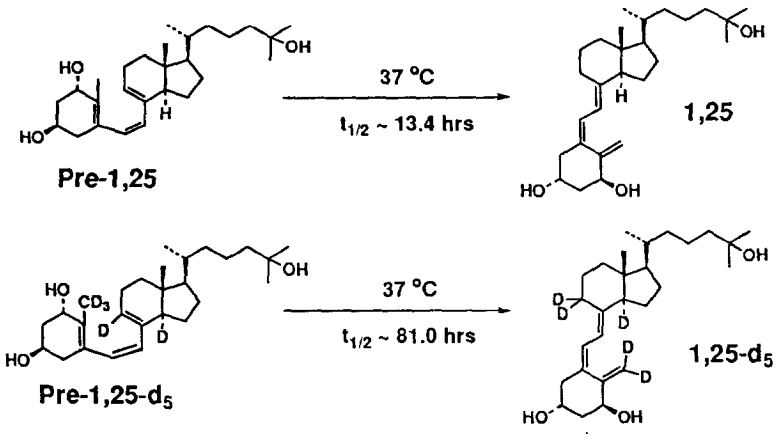
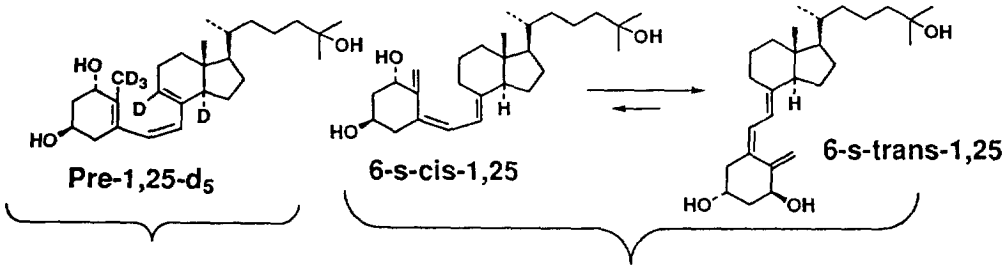


Fig. 7. Isomerization of pre-1,25 to 1,25 and of pre-1,25-d_5 to 1,25-d_5.

terium in pre-1,25-d_5 by slowing down its spontaneous isomerization via a [1,7]-H shift facilitates determination of its intrinsic biological properties. Thus, it is less prone to isomerize to 1,25-d_5, leaving open the possibility that the observed activity is due to the latter rather than the former.

The hormone 1,25 induces biological responses via non-genomic (e.g. by binding to the putative membrane 1,25 receptor in the intestine, m-VDR, which then induces Ca^{2+} channel opening and then the rapid intestinal calcium transport response known as transcaltachia) and genomic responses (e.g. by binding to the nuclear 1,25 receptor in the intestine, n-VDR, which mediates protein synthesis and then intestinal calcium transport). With pre-1,25-d_5 and 1,25-d_5 as well as their unlabeled counterparts in hand, the remarkable observation [11] was made that whereas 1,25 is significantly more active than pre-1,25-d_5 in assays reflecting genomic responses, the latter was equally as active as 1,25 in assays for non-genomic effects. From Fig. 8, which delineates the various assay systems examined, and the assumption that pre-1,25-d_5 does not isomerize to 1,25-d_5 under the assay conditions, two proposals were suggested: first, since pre-1,25-d_5 and 1,25 are equally active in producing non-genomic biological action, 6-s-*cis*-1,25 is the active conformer in eliciting non-genomic effects with pre-1,25 behaving simply as an excellent analog of this conformer; second, since pre-1,25-d_5 exerts only weak genomic responses as compared to 1,25, and since pre-1,25-d_5 cannot assume the extended, non-steroidal 6-s-*trans*-1,25 topology, the latter shape is required for genomic effects.

It is the purpose of this paper to more rigorously evaluate this interpretation (that genomic effects require an extended, non-steroidal 6-s-*trans*-1,25 topology and that non-genomic effects require the steroidal 6-s-*cis*-1,25 topology) by detailing recent advances in the structural analysis of 1,25 and by describing some recent structure–function studies.



1) Pre-1,25-d_5 & 1,25 equivalently active in several non-genomic assay systems.

Transcaltachia (perfused chick intestine)
$^{45}Ca^{+2}$ uptake through voltage-gated Ca^{2+} channels (ROS 17/2.8 cells)

2) Pre-1,25-d_5 less active than 1,25 in several genomic assay systems.

In vitro binding to Vit D binding protein (human plasma)
In vitro binding to intestinal nuclear 1,25 receptor, n-VDR (chick, pig)
Induction of serum osteocalcin and calcium (chick)
Inhibition of cellular proliferation and osteocalcin induction (MG-63 cells)
Induction of cellular differentiation (HL-60 cells)

Fig. 8. Non-genomic versus genomic actions of pre-1,25-d_5 and 1,25.

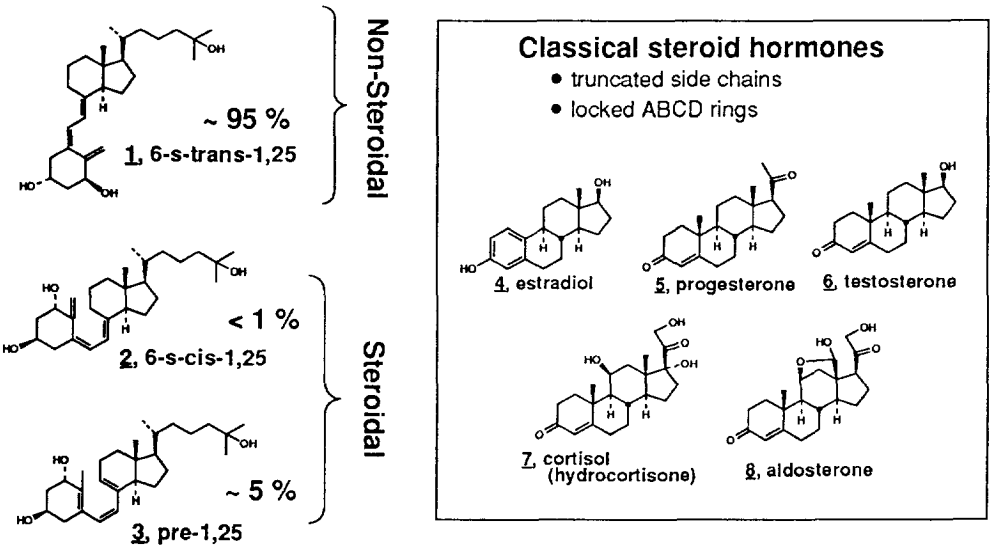


Fig. 9. A comparison of the shape of 1,25 (steroidal or non-steroidal) and pre-1,25 (steroidal) versus the classical steroid hormones.

THE STEROIDAL (6-s-*cis*) AND NON-STEROIDAL (6-s-*trans*) OR EXTENDED) FORMS OF 1,25

Based on ^{1}H-nuclear magnetic resonance (NMR) coupling constants, at equilibrium (37°C), the free ligand 1,25 in solution consists of 95% of the 6-s-*trans* conformer wherein the intercyclic diene component (C5=C6—C7=C8) of the vitamin D triene chromophore is approximately planar [12]. The dihedral angle of the 6,7-single bond cannot be estimated very precisely by NMR spectroscopy, but X-ray crystallographic data (for a review, see reference [4]) indicates that the conformation about the s-*trans*-6,7 single bond is twisted out of plane by an average of ±8.5° with a range of 3–16° for seven separate determinations. The dihedral angle for the 5,10 single bond of the bis-exocyclic diene component (C6=C5—C10=C19) of the vitamin D triene chromophore, which is locked in an s-*cis* conformation by virtue of its location in the A-ring, is severely distorted from planarity by an average of ±56° with a range of 46–57°. It should be noted too that the degree and direction of 5,10 single bond distortion from planarity differs for the two different chair conformers which the A-ring can assume. The extent to which crystal packing forces contribute to the range of values obtained is unclear. An interesting result in comparing these crystallographic structures is that opposite A-ring chair forms or both chair forms in a single structure determination have been observed [13, 14]. It has however long been known that in solution both A-ring chair forms exist in rapid equilibrium with one another and, in general, their relative proportions are similar [15, 16]. Moreover, in vitamin A compounds, in which its ring-side chain connection (also coincidentally numbered as the 6,7-single bond) may also be s-*cis* or s-*trans*, structural results have been obtained for participation by both kinds of conformers (twisted s-*cis* and planar s-*trans* forms have both been observed) [17].

Computational and experimental studies of 1,3-butadiene all agree that its s-*trans* conformer is planar; this can be compared to the out-of-plane distortion of ±8.5° of the s-*trans* rotamer of vitamin D (i.e. the 6,7 single bond). Similar studies of the less stable s-*cis* conformer of 1,3-butadiene has been a topic of some controversy, with suggestions ranging from a planar structure to gauche arrangements (a non-planar, twisted conformation); a recent view seems to suggest that the latter is probably correct [18]. This can be compared to the ±56° out-of-plane value cited above for the 5,10 single bond in the vitamin D A-ring. Several matters need to be emphasized. (1) The overall structure in the solid state, in solution, or in the gas phase, determined experimentally or computationally, of various vitamin D metabolites or analogs which have been examined by X-ray crystallography and other physical techniques is not very different from that previously surmised (depicted qualitatively in panel A of Fig. 6). (2) Rotations about carbon–carbon single bonds are fast and there is no question that the 6,7-single bond of vitamin D can assume the s-*cis* (planar or twisted arrangement) to at least some degree. As summarized in Fig. 9, if 1,25 is allowed to thermally equilibrate, about 95% of 1,25 exists in the non-steroidal, extended 6-s-*trans*-1,25 form and about 5% in the double bond shifted pre-1,25 form, which because of the Z or *cis* double bond across C6–C7, exists in a steroidal shape [4, 9]. For 1,25 itself however, it is estimated that 98.8% exists as 6-s-*trans*-1,25 and 1.2% as 6-s-*cis*-1,25 (unpublished observations; other laboratories have calculated that ≥5–12% of s-*cis* conformer exists in equilibrium with 88– ≤95% of the s-*trans* form [19, 20], and a still earlier estimate favor-

ing the s-*cis* conformation has been reported [21]); the s-*cis* conformer, however, has thus far not been detected directly, either spectroscopically or by other methods. By 6-s-*cis*, we mean the family of conformers that reside within $\pm 90^{\circ}$ of the planar s-*cis*-conformer, which as far as is apparent, does not exist as an energy minimum (likewise, by 6-s-*trans*, we mean the family of conformers that reside within $\pm 90^{\circ}$ of the planar s-*trans* conformer). Experimental evidence that the 6-s-*cis*-1,25 must exist is based on the view that 1,25 undergoes thermal equilibrium with pre-1,25; this equilibrium interconversion involves the well established [1,7]-H shift, which requires a cyclic transition state, possible for 6-s-*cis*-1,25 but impossible for 6-s-*trans*-1,25 [9, 22]. (3) Finally, it can be said that since spectroscopically undetected, but kinetically competent concentrations of 6-s-*cis*-1,25 exists in rapid equilibrium with the dominant 6-s-*trans*-1,25, it is completely reasonable to expect that upon binding of 1,25 to protein receptors, the ligand may exist entirely as 6-s-*cis*-1,25. As is now well known, the receptor gene for 1,25 belongs to the same superfamily of *trans*-activating regulators of gene-transcription which includes the receptors of the classical steroid hormones shown in Fig. 9 as well as the retinoid and thyroid hormones (not shown) [23, 24]. A comparison of the classical steroid hormones (with their fully intact ABCD fragments) with the steroidal versus non-steroidal forms of 1,25 suggests almost intuitively that 1,25 ought to possess a classical steroid shape when bound to its receptors. This intuitive view can be asked in reverse: can a B-seco form of one of the classic steroid hormones still bind to its own receptor? Interestingly, the answer is yes! Recently, DeLuca and associates reported a seco-B-ring pregnacalciferol analog (with the same triene chromophore as 1,25) which bound significantly to the progesterone receptor [25].

Figure 10 presents the structure of the now familiar 6-s-*trans*-1,25, and seeks to depict in 3-dimensional fashion the rotation of its 6,7-single bond through a 360° cycle (the C10–C19 double bond as well as the C3β-OH group is deleted to simplify the drawing). Although there is a continuum of 6,7-single bond rotamers of various torsional energies, "snapshots" of the selected conformers at 90° increments are presented, starting with the "planar-s-*trans* conformer". While holding the CD/side chain (SC) in place, a 90° rotation (step 1) in a downward direction places the A-ring towards the under-side of the CD fragment with this ring approximately in the plane of the drawing, labeled as the α-conformer. A second 90° rotation (step 2) continuing in the same direction produces the "planar-s-*cis* conformer"; a third 90° rotation (step 3) again orients the A-ring in the plane of the drawing, but now this ring is above the CD plane (labeled as the β-conformer). A final 90° rotation (step 4) regenerates the starting "planar-s-*trans* conformer".

Within this 360° cycle are numerous energy minima and molecular mechanics computations provide a convenient method to identify these minima in a more realistic manner (Fig. 11); it should of course be clear that the structures in Fig. 10 depict the 1,25 rings as planar for simplicity, rather than in the more realistic puckered chair like conformers or other staggered arrangements of the C–C single bonds. In Fig. 11, the lowest energy (global minimum) conformer of 1,25 (with an isopropyl side chain as a model to reduce

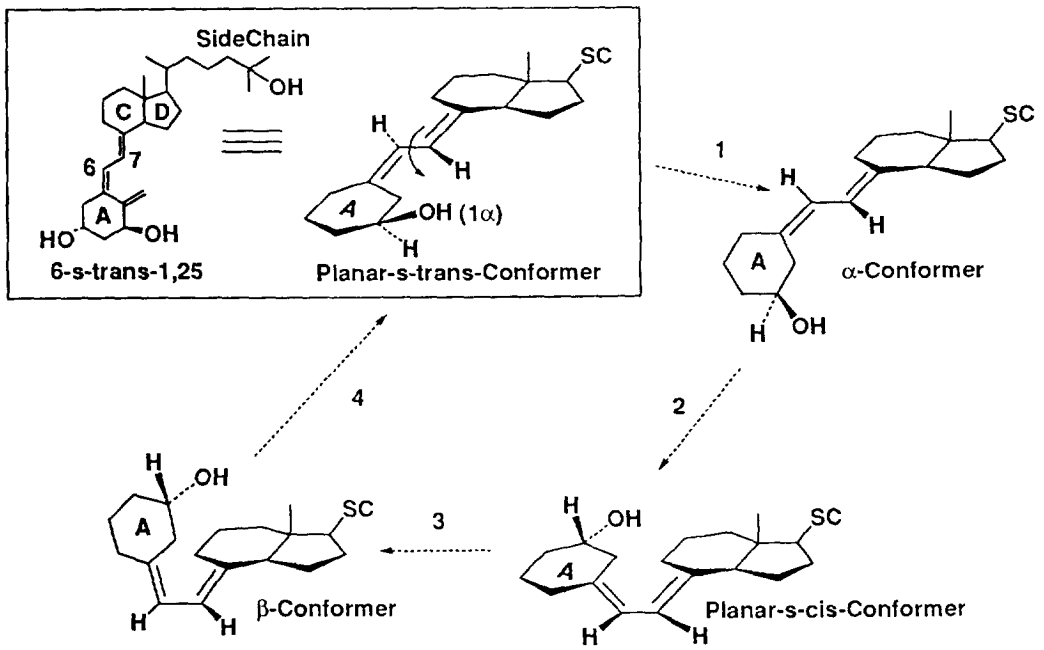


Fig. 10. On the 360° rotation of the 6,7-single bond of 1,25. In these stereoviews, the 1,25 molecule is drawn with the A- and CD-rings flat (the C3 hydroxyl and the C10–C19 double bond were left off for clarity).

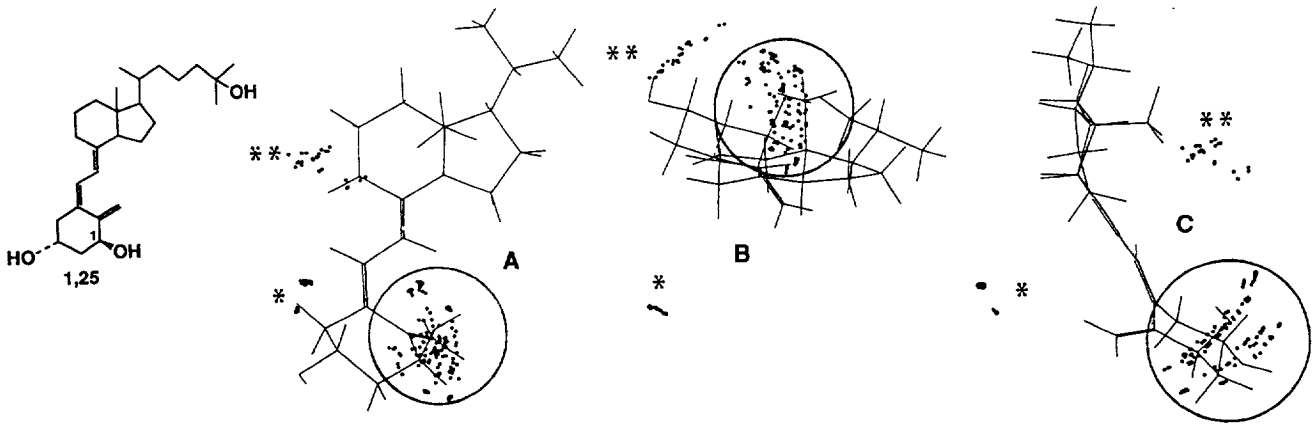


Fig. 11. Three different views of dot maps for 1,25 derived from molecular mechanics computations. The line drawings in panels A, B and C depict the global energy minimum conformation and the dots represent the excursions of the C1-hydroxyl of 1,25 for the various local minimum conformers.

computational time) is given as line drawings in three different orientations in panels A, B and C. To generate the dot maps, portions of the D-ring and side chain of the higher energy conformers were then overlaid on the global minimum and then the location in space of the higher energy conformers was represented simply by the location of the C1 oxygen atom of the A-ring, depicted as a dot as shown in panels A, B and C. This dot map technique has previously been described by this laboratory in an analysis of the side chain [4, 10]. Three clusters of dots emerge in these maps wherein the circled set represents the excursions of the C1-oxygen of 6-s-*trans*-1,25 for its various local minimum energy conformers (which not surprisingly includes the global minimum form). The circled set, which includes both chair forms of the A-ring, represents about 98.8% of the conformations available to 1,25 within a ~4 kcal/mol window of the global minimum. Two other clusters, indicated by an asterisk (*) and a double asterisk (**), represent the family of twisted 6-s-*cis*-1,25 conformational minima and these constitute 1.2% of the conformers available to 1,25 within this same energy window. The 6-s-*cis* conformers among those in cluster *, having the C10(19) double bond positioned on the under-side (α-face) of the CD ring, are somewhat more stable than those 6-s-*cis* conformers in cluster **, having this same double bond on the β-face of the CD ring. Finally, some of the conformers in cluster * and ** have the A-ring nearly perpendicular to the CD rings, akin to the α- and β-conformers shown in Fig. 10. Several matters need to be delineated before leaving this topic. (1) The rotations depicted in Fig. 10 leading to the energy minimized structures of Fig. 11 are extremely rapid. 1,25 is an inherently flexible molecule and in no sense whatever can one speak of a biologically active conformer from this analysis of free ligand. (2) Whether these computations represent accurate descriptions of the 3-dimensional structure of the various energy minimum conformers available to 1,25 remains as of yet uncertain. There certainly remain serious questions concerning whether the molecular mechanics programs (such as PC Model) used here can provide accurate structural information [4, 10]. (3) The latter may not be very significant since the goal is to develop an understanding of the structure of the ligand (1,25) in the active site of receptor. While it is likely that 1,25 binds in a reasonably stereospecific orientation with receptor, the actual topology of 1,25 in the active site may not be any one of the energy minimum structures even if these minima are accurate representations. This matter has been well emphasized and has been referred to as the "rusting of the lock and key model for protein-ligand binding" [26]. (4) The most practical approach at this stage to understand the structural demands for the various biological activities characteristic of 1,25 is to prepare a set of analogs which might mimic approximately one or more of the continuum of conformers generated by conformational rotations (such as those depicted in Figs 10 and 11). Certainly, efforts need to be made to develop direct methods of ascertaining the structure and conformation of the various ligand–receptor entities, but these remain targets for future research efforts.

The remainder of this paper seeks to describe progress in studies directed towards developing conformationally locked mimics of the various conformers which 1,25 can assume. The area of focus is to mimic approximately a single, instantaneous conformation which 1,25 can assume through the 360° cycle depicted schematically in Fig. 10.

CONFORMATIONALLY LOCKED MIMICS OF 6-s-*cis*-1,25

To further test the hypothesis that the high non-genomic biological activity of pre-1,25 can be attributed to its excellent ability to mimic 6-s-*cis*-1,25 (Fig. 12, top panel), and not due to prior isomerization of pre-1,25 to 1,25 (6-s-*cis* or 6-s-*trans*), a second generation of 6-s-*cis* analogs, namely the 4 provitamin

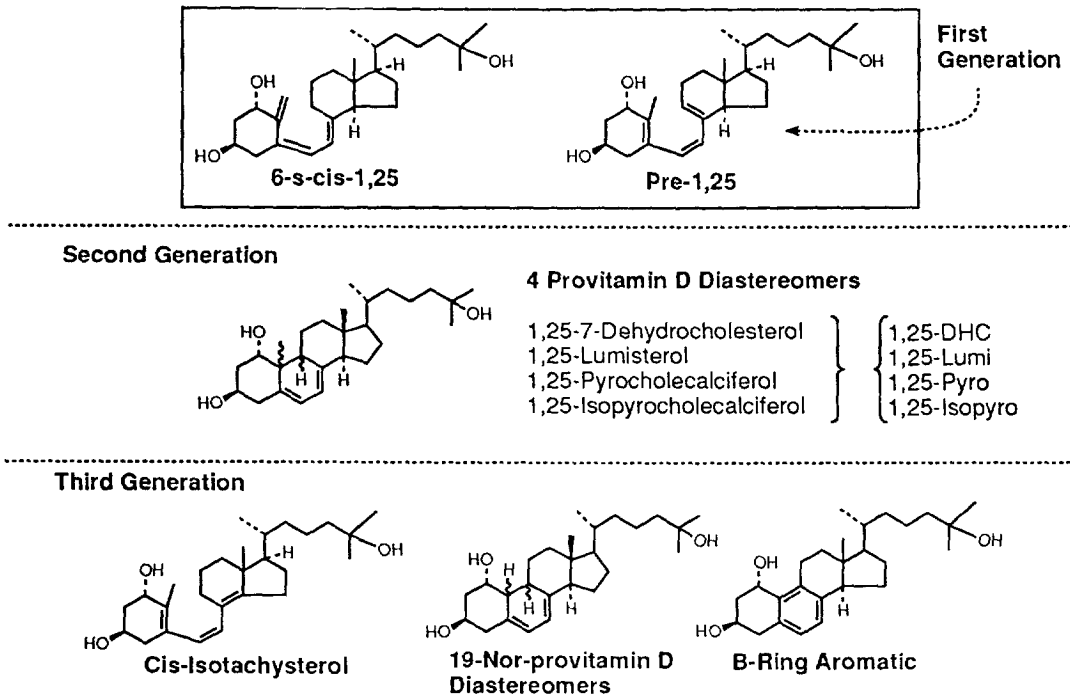


Fig. 12. First, second and third generation 6-s-*cis* locked analogs of 6-s-*cis*-1,25.

D diastereomers, were synthesized (Fig. 13) and biologically evaluated [27]. Although assessment of the complete biological profile of these analogs remains incomplete, 1α,25-$(OH)_2$-lumisterol$_3$ (1,25-Lumi) has been established to be equivalently active in eliciting the non-genomic activity of transcaltachia as pre-1,25 and 1,25. Moreover, 1,25-Lumi binds as effectively as 1,25 itself to the basal lateral membrane (m-VDR) considered to induce transcaltachia [28]. Thus, since 1,25-Lumi is highly unlikely to isomerize thermally (like pre-1,25) to 1,25, excellent support for the involvement of 6-s-*cis*-1,25 in mediating a vitamin D response is in hand. Both in terms of transcaltachia and binding to m-VDR, 1α,25-$(OH)_2$-7-dehydrocholesterol (1,25-DHC) is less active than 1,25-Lumi; thus, there is a significant level of stereoselectivity in mediating these non-genomic responses.

ONGOING AND FUTURE STUDIES

Computational modeling of 1,25-Lumi and 1,25-DHC as well as 1α,25-$(OH)_2$-pyrocholecalciferol (1,25-Pyro) and 1α,25-$(OH)_2$-isopyrocholecalciferol (1,25-Isopyro) reveals a resemblance of 1,25-Lumi and 1,25-Pyro wherein both their C19 angular methyl groups reside below the steroid plane as normally viewed (because of their 10α-stereochemical configuration), but it remains for future experiments to develop their complete structure–function relationships.

Experiments are also underway to biologically evaluate several third generation analogs, which have recently become available (bottom panel, Fig. 12). *Cis*-isotachysterol$_3$ (*cis*-Iso-T) very closely resembles pre-1,25 in that both are 6-s-*cis* locked and both are

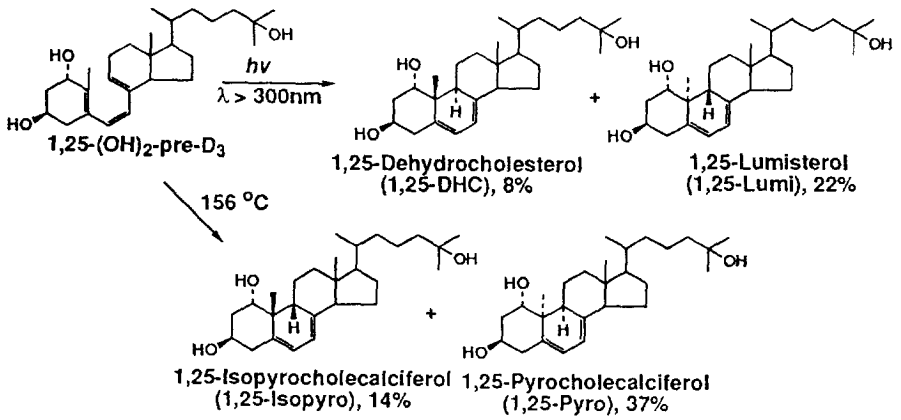


Fig. 13. Synthesis of the four diastereomeric 1α,25-dihydroxyprovitamin D_3s from pre-1,25.

seco-B steroids (the C9–C10 bond is broken); however, *cis*-iso-T is incapable of rearranging to 1,25.

The 19-nor-provitamin D diastereomers completely parallel in structure the provitamin D diastereomers (middle panel, Fig. 12), except that the C19-angular methyl group has been removed. These 19-nor analogs should be useful in probing steric effects attributable to the methyl group at C19 in the parent provitamins, which should in turn be compared to the nearly A-ring-co-planar methyl or methylene groups present in pre-1,25 and 6-s-*cis*-1,25, respectively.

In analyzing the analogs given in Fig. 12, it is useful to compare their conformations with the continuum of conformers available to 1,25 shown earlier in Fig. 10. Pre-1,25, *cis*-Iso-T, and the various provitamins and 19-nor-provitamins (Fig. 12) appear to mimic conformations intermediate between the α-conformer and the planar-s-*cis* conformer or between the latter and the β-conformer (Fig. 10). The B-ring aromatic analog (Fig. 12) is considered to be a reasonable representative of the planar-s-*cis*-conformer of 1,25 (Fig. 10), the latter being what should be the highest energy conformational species among the continuum of triene conformers available to 1,25. As for possible conformational mimics of the α- and β-conformers of Fig. 10, wherein the A-ring (including C6) is mutually perpendicular to the approximate plane defined by the CD ring, 1,25-vinylallenes (the β-conformer mimic is shown in Fig. 4) in appropriate stereochemical forms have recently been synthesized by this laboratory [6]. The latter vinylallenic analogs and those analogs mentioned earlier (Fig. 12) are presently undergoing exhaustive scrutiny.

CONCLUDING REMARKS

For the non-genomic calcium transport response of transcaltachia, the biological results obtained for pre-1,25 and the pro-1,25 analogs point strongly to the involvement of the 6-s-*cis* conformer of 1,25. This represents a clear case of biological action occurring via a less stable, ground state conformer. There is a continuum of 6,7-single bond conformers available to 1,25 and caution should should be exercised in assigning a specific conformer to be the "biologically active conformer" based on free ligand topology alone. Rotational isomerism is too facile and since there is a conformational continuum, free ligand conformational minima (local or global) may have little to do with the manner in which 1,25 is bound to receptor. For the genomic calcium transport response, and for other genomic (or non-genomic) effects, there is no clear evidence whether the steroidal (s-*cis*) or non-steroidal (s-*trans*) conformer of 1,25 is involved. We have perhaps over emphasized the suggestion that the genomic response of calcium transport mediated by a nuclear 1,25-receptor (n-VDR) involves 6-s-*trans*-1,25 [11, 27]. The evidence has been based on the negative

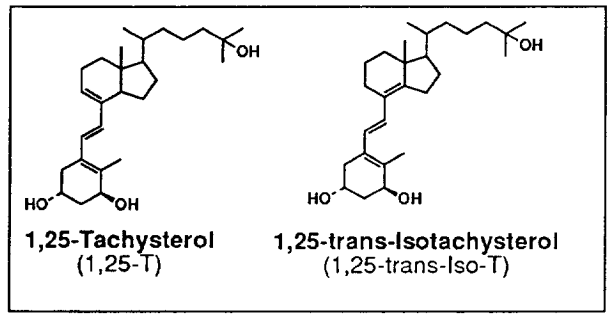


Fig. 14. The first generation 6-s-*trans* locked analogs of 6-s-*trans*-1,25.

observation that pre-1,25 and 1,25-Lumi exhibit rather weak genomic activities [11]. In order to address this matter further, it would be desirable to evaluate 6-s-*trans* locked analogs of 1,25 which might mimic either the planar 6-s-*trans*-1,25 or some intermediate conformer between the latter and either the α or β-conformer in Fig. 10. Two such analogs are shown in Fig. 14, namely 1α,25-$(OH)_2$-tachysterol (1,25-T) and 1α,25-$(OH)_2$-trans-isotachysterol (1,25-trans-iso-T). In preliminary findings, we have determined that 1,25-T exhibits an even lower ability to elicit genomic effects (e.g. binding to the nuclear receptor and the *in vivo* intestinal calcium transport response) than pre-1,25 or 1,25-Lumi. Although the genomic actions of the latter pair of analogs are low, this may simply reflect that they are not good mimics of the putative, "biologically active conformer" of a 6-s-*cis*-1,25; by contrast, pre-1,25 and 1,25-Lumi have serendipitously turned out to be excellent mimics of the "active conformer" of 6-s-*cis*-1,25 involved in mediating transcaltachia. The possibility cannot be excluded that all vitamin D steroids exert their genomic as well as non-genomic responses through s-*cis*-steroid-like conformations, perhaps twisted in different ways among the continuum of 6,7-single bond conformers.

Acknowledgement—This work was supported in part by USPHS grants DK-16,595 (WHO) and DK-09012 (AWN).

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