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Research Paper

Drug design with a new transition state analog of the hydrated carbonyl: silicon-based inhibitors of the HIV protease

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Abstract

Background: Silicon is the element most similar to carbon, and bioactive organosilanes have therefore been of longstanding interest. Design of bioactive organosilanes has often involved a systematic replacement of a bioactive molecule's stable carbon atoms with silicon. Silanediols, which are best known as unstable precursors of the robust and ubiquitous silicone polymers, have the potential to mimic an unstable carbon, the hydrated carbonyl. As a bioisostere of the tetrahedral intermediate of amide hydrolysis, a silanediol could act as a transition state analog inhibitor of protease enzymes.

Results: Silanediol analogs of a carbinol-based inhibitor of the HIV protease were prepared as single enantiomers, with up to six stereogenic centers. As inhibitors of this aspartic protease, the silanediols were nearly equivalent to both their carbinol analogs and indinavir, a current treatment for AIDS, with low nanomolar K_i values. IC_{90} data from a cell culture assay mirrored the K_i data, demonstrating that the silanediols can also cross cell membranes and deliver their antiviral effects.

Conclusions: In their first evaluation as inhibitors of an aspartic protease, silanediol peptidomimetics have been found to be nearly as potent as currently available pharmaceutical agents, in enzyme and cell protection assays. These neutral, cell-permeable transition state analogs therefore provide a novel foundation for the design of therapeutic agents. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Drug design; HIV; Organosilane; Protease inhibitor; Silanediol

1. Introduction

Proteolytic enzymes mediate many key biological events by hydrolyzing specific peptide bonds (**1** → **3**+**4**), and have thereby achieved a prominent position in the hierarchy of pharmaceutical targets [1]. Inhibitors of angiotensin-converting enzyme currently play a major role in the control of hypertension [2], and inhibitors of the HIV protease have proven to be potent tools for the control of AIDS [3,4]. Proteases are intimately involved in cancer [5] and apoptosis [6,7], and their effects are manifest in diseases as diverse as anthrax [8,9], cystic fibrosis [10], and Alzheimer's disease [11]. Efficacious in vivo inhibitors for many of these targets, however, remain elusive.

At the center of protease inhibitor design, the first step in creation of a new therapeutic agent is selection of a nonhydrolyzable analog of **2**, the tetrahedral intermediate of amide hydrolysis (Fig. 1). Enzyme binding of the energetically disfavored **2**, through Coulombic interactions, solvation, and hydrogen bonding, results in dramatic accelerations in the rate of hydrolysis of **1** [12]. Nonhydrolyzable isosteres of **2**, when bound at the catalytic site of the enzyme, inhibit proteolysis and can thereby eliminate the biological consequences of that proteolytic action.

The diversity of inhibitor designs available to the medicinal chemist, however, is limited by the small number of unique isosteres of **2**. For aspartic proteases, these isosteres include carbinols, ketones, and phosphinic acids (**5**–**7**), each with its characteristic attributes of size, shape and charge distribution. Testing of compounds containing

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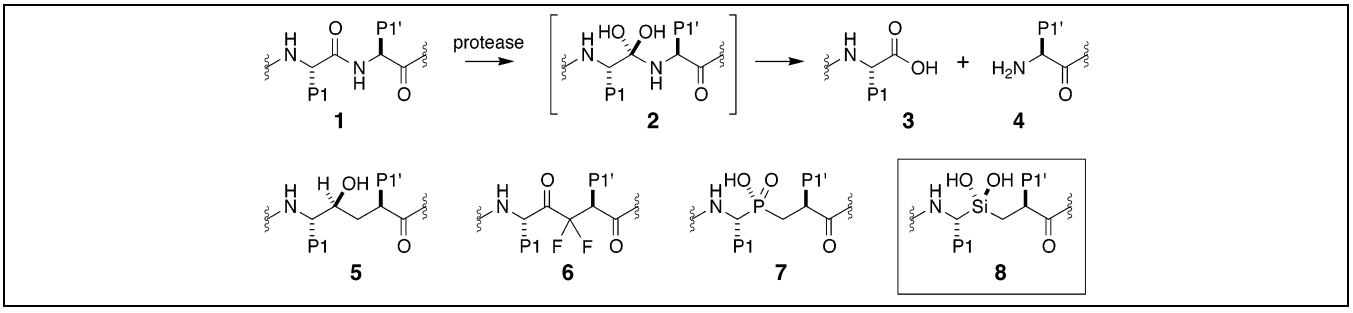


Fig. 1. Tetrahedral intermediate **2** can be replaced by nonhydrolyzable **5**–**7**, resulting in protease inhibitors. New analog **8**.

these isosteres against the HIV protease have found only carbinols to be highly effective [3,13,14]. Silanediols are well known chemical structures, but silanediols such as **8** have never been prepared. This may be because simpler silanols have been found to be ineffective as inhibitors of hydrolase enzymes [15,16], as well as the general knowledge that silanediols readily polymerize [17]. We describe here the first example of a silanediol designed as an aspartic protease inhibitor, the demonstration of its effectiveness against the HIV protease and its ability to suppress the virus in whole cell assays.

2. Results

Silicon is the element most similar to carbon but differs dramatically from carbon in its ability to accommodate double bonds. The carbon–oxygen double bonds in **1** are very strongly favored over the hydrated form **2**. As a point of comparison, the carbon–oxygen double bond of acetone **9** (Fig. 2, X = C) is more readily hydrated than **1** but remains less than 0.01% hydrated **10** in aqueous solution. In contrast, formation of a silicon–oxygen double bond is extremely disfavored over its hydrate, the silanediol **10** (X = Si). A silanediol can therefore be considered to be an analog of the unstable hydrated carbonyl **2**.

The simplest and best known silanediol, dimethylsilanediol **10** (X = Si), is intrinsically unstable toward dehydration/polymerization, forming permethylsilicone **11**. Approximately one million tons of this silanediol are synthesized each year to make silicone polymers [18]. More sterically hindered silanediols *are* stable, but unlike the silanediols reported here, the known silanediols are composed solely of simple alkyl and aryl groups [19].

Silanediol-based dipeptide analogs like **8** are new chemical species, more complex than any silanediol previously described. We expected that the steric shielding of the organic substituents in **8** would disfavor formation of oligomers. Silanols are equivalent to carbinols like **5** in their capacity to accept hydrogen bonds, and are better hydrogen bond donors [20]. These are ideal attributes for interaction with the active site of aspartic proteases, where two carboxylic acid residues stabilize intermediate **2** through hydrogen bonding. To assess the effectiveness of silanediols as aspartic protease inhibitors, we have prepared the C_2 symmetric **17** (Fig. 3), an analog of the potent HIV protease inhibitor, carbinol **18** [21].

Our synthetic approach to silanediol **17** utilized diphenylsilane **15** as a key intermediate, unveiling the potentially polymerizable silanediol group by acid-catalyzed hydrolysis of the aryl–silicon bonds in the last synthetic step. Acidic aryl silane hydrolysis was anticipated to be compatible with the surrounding functionality since similar conditions have been used for polypeptide deprotection [22]. Synthesis of **15** began with enantiomerically pure iodide (*S*)-**12** [23], which was converted to the lithium reagent **13** by metal–halogen exchange [24,25]. Coupling of this lithium reagent with slightly less than one-half of an equivalent of dichlorodiphenylsilane gave the corresponding (*S,S*)-dialkyldiphenylsilane in 98% yield. Cleavage of the benzyl ethers with boron tribromide led to the diol **14** (93%). Oxidation of the diol to the dialdehyde using Swern conditions [26], followed by oxidation with potassium permanganate [27], gave the diacid in 94% yield for two steps. Diamides **15** were formed by coupling the diacid with benzyl amine and with 1-(*S*)-2-(*R*)-1-amino-2-indanol using diethyl phosphorocyanidate as an activating agent [28] to give diamides **15a** and **15b**, isolated in 62% and 75% yields, respectively.

The critical deprotection of diphenylsilanes **15a** and **15b** was performed with an excess of trifluoromethanesulfonic acid (triflic acid). This transformation can be followed by ^{1}H NMR spectroscopy and involves a symmetric intermediate, believed to be spirocycle **16**. Intermediate **16** would be formed by amide-assisted cleavage of the phenyl–silicon bonds. This intermediate is hydrolytically unstable [29] and treatment with ammonium hydroxide provided the desired silanediols **17a** and **17b** as colorless solids in 40–80% yield after purification.

Table 1 shows the results from evaluation of silanediols

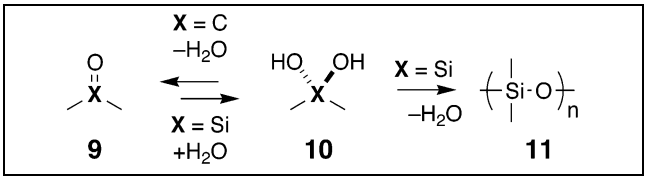


Fig. 2. Carbon, but not silicon, forms strong double bonds to oxygen.

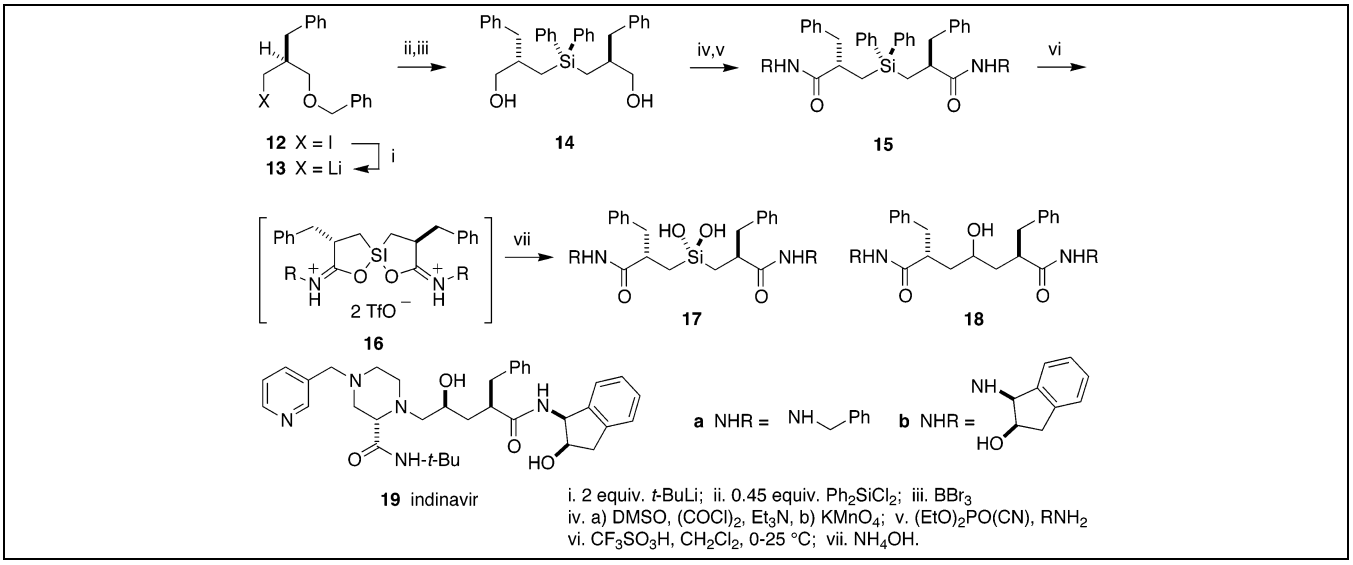


Fig. 3. Synthesis of silanediols **17a** and **17b**.

17a and **17b**, and carbinols **18a** and **18b** as HIV protease inhibitors using an assay described previously [30], with indinavir **19** [31] as a benchmark. The two benzyl amides, silanediol **17a** and carbinol **18a**, gave identical, low micromolar K_i values, indicating a significant level of enzyme binding. The equivalence of these inhibitors established, for the first time, that silanediols can be as effective as carbinols [13], the central component of all commercial aspartic protease inhibitor drugs.

The use of the (1*S*,2*R*)-1-amino-2-indanol as amide groups in **17b** and **18b** dramatically enhanced the enzyme inhibition, with the K_i for carbinol **18b** lowered by nearly 10^4 compared to **18a**. Silanediol **17b** binds to the enzyme nearly as well as the carbinol **18b**, with a K_i of 2.7 nM. The binding data for both **17b** and **18b** compare very favorably with the established HIV therapeutic agent indinavir **19**, which has a K_i of 0.37 nM in this assay.

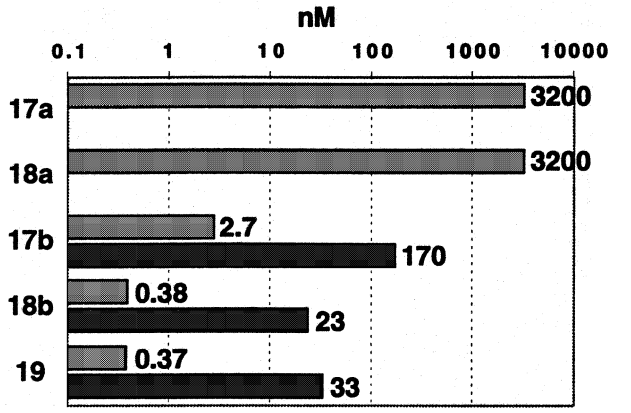
Cell culture is a more rigorous evaluation of therapeutic potential, because antiviral activity can be demonstrated only after the inhibitor crosses cell membranes. The IC_{90} values in Table 1 are the concentration of compound causing a 90% reduction in virus replication relative to untreated control infections [32]. For silanediol **17b** and carbinols **18b** and **19**, this IC_{90} value parallels the enzyme K_i values. Addition of serum protein to these assays, to probe the effect of nonspecific protein binding that can impact bioavailability in vivo [33], does not alter the relative efficacy of the silanediol as an antiviral agent.

The consistency of these data is remarkable. Comparing the K_i values for **17b** and **18b**, the carbinol **18b** is more active than the silanediol **17b** by a factor of 7.1, while the IC_{90} values differ by a factor of 7.4. Silanediol **17b** contains the same skeleton as carbinol **18b**, the latter being highly optimized for the HIV protease active site [21]. The seven-fold difference in activity between silanediol **17b** and carbinol **18b** may be due to the size of the silicon atom in the inhibitor backbone. The silicon–carbon bond is 20% longer than a carbon–carbon bond (1.88 Å vs. 1.54 Å). Three-dimensional structures for the tetrahedral intermediate (A), the hydroxyethylene group (B), and the silanediol (C) are shown in Fig. 4. Not only is silanediol C larger than A and B, it has a more pronounced surface charge distribution that could accentuate Coulombic and dipolar interactions at the active site.

3. Significance

The silanediol functional group is a new bioisostere of the hydrated amide carbonyl. It is neutral, cell-permeable, and the outstanding hydrogen bonding of silanols allows for good interactions with the active site of aspartic pro-

Table 1
Enzyme inhibition (K_i, light bars) and cell protection (IC_{90}, dark bars) data for silanediols **17**, and carbinols **18**, and indinavir **19**



K_i data were determined using assay system C described in [30]. IC_{90} antiviral activity was determined in a cell-based system described in reference [32].

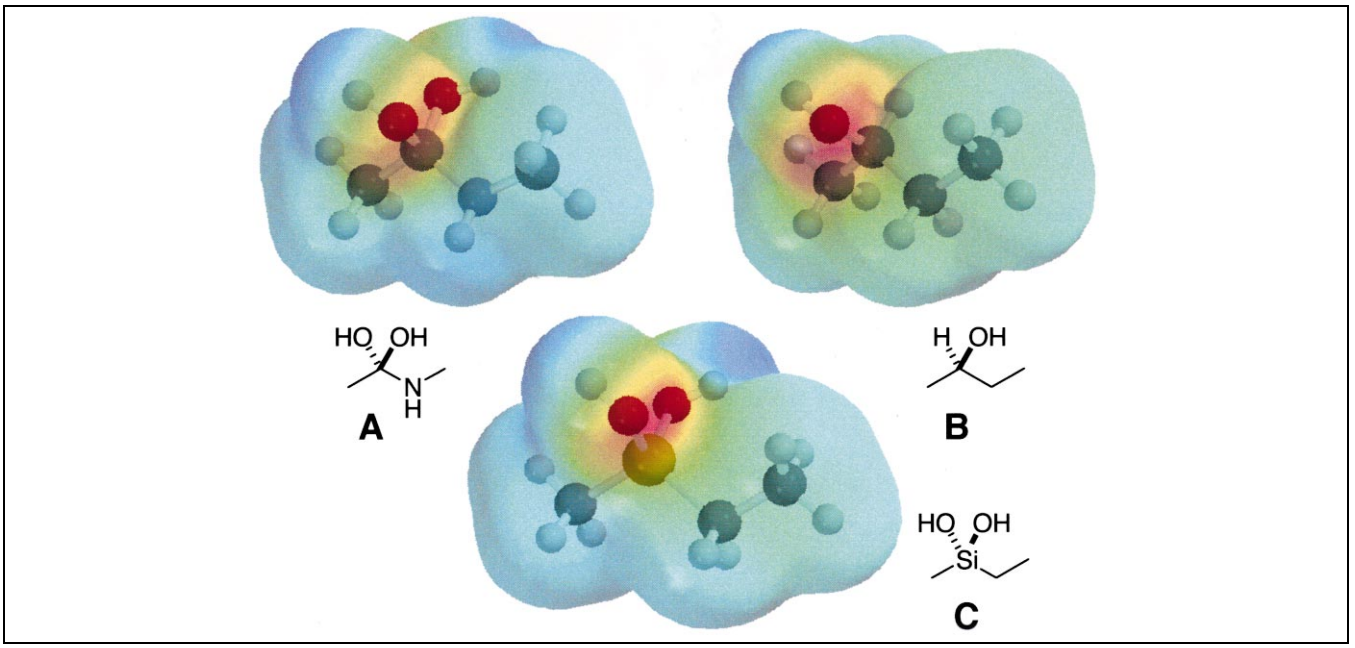


Fig. 4. Molecular models of the tetrahedral intermediate of peptide hydrolysis A, the classic carbinol isostere B and the new silicon-based analog C represented as ball and stick models inside the molecular surfaces. Surface colors indicate the calculated electron densities. These structures were calculated using the semiempirical method PM3, with geometry optimization, using MacSPARTAN (Wavefunction, Irvine, CA, USA).

teases. The efficacy of a silanediol as an HIV protease inhibitor, at concentrations within an order of magnitude of the therapeutically effective agent indinavir, demonstrates for the first time that a silicon-based transition state analog can be a centerpiece of aspartic protease inhibitor design. In conjunction with our recent report of an effective silanediol-based metalloprotease inhibitor [34], it is clear that silanediols can be potent and generally applicable inhibitors across several protease enzyme classes. Moreover, the effective antiviral properties of silanediol **17b** in cell culture indicate that these structures have the potential to be the basis of new pharmaceutical agents. The competency of these silanediol inhibitors contrasts with previous reports in which silanediol [15] and silanetriols [16] did not inhibit hydrolase enzymes, and is also at variance with the perception that silanediols are unstable, problematic chemical entities. Extending the application of silanediols to the inhibition of other proteases and other protease classes will serve to further define the breadth of this new medicinal chemistry tool, and will be reported in due course.

4. Materials and methods

*4.1. N,N′-Dibenzyl 2-(S)-6-(S)-dibenzyl-4,4-diphenyl-4-sila-heptane-diamide (**15a**)*

To a solution of 2-(*S*)-6-(*S*)-dibenzyl-4,4-diphenyl-4-sila-heptanedioic acid (228 mg, 0.45 mmol) in DMF (5 ml) at 0°C was consecutively added benzylamine (0.12 ml, 1.13 mmol), DPPA (0.24 ml, 1.13 mmol), and triethylamine (0.28 ml, 2.03 mmol). The reaction mixture was stirred at 0°C for 2 h and then warmed to room temperature overnight. The mixture was diluted with 10 ml ethyl acetate and washed successively with 5% aqueous HCl, water, saturated aqueous sodium bicarbonate, and saturated aqueous NaCl. After drying over $MgSO_4$ and filtering, the solution was concentrated in vacuo. Flash chromatography (7:3 hexane/ethyl acetate) afforded **15a** as a foam (189 mg, 62%). $R_f = 0.26$ (7:3 hexane/ethyl acetate); mp 55–60°C; ^{1}H NMR ($CDCl_3$) δ 7.47–6.79 (m, 30 H), 5.56 (t, 2 H), 4.31 (dd, 2 H, $J = 14.7$ Hz and 6.7 Hz), 3.82 (dd, 2 H, $J = 14.7$ Hz and 4.5 Hz), 2.87 (dd, 2 H, $J = 13.2$ Hz and 10.2 Hz), 2.55 (dd, 2 H, $J = 13.2$ Hz and 4.7 Hz), 2.42 (m, 2 H), 1.68 (dd, 2 H, $J = 15.0$ Hz and 7.4 Hz), 1.48 (dd, 2 H, $J = 15.0$ Hz and 6.6 Hz); ^{13}C ($CDCl_3$) δ 175.2, 139.8, 137.7, 135.4, 134.7, 129.7, 128.9, 128.4, 128.3, 128.1, 127.6, 127.2, 126.2, 45.3, 43.2, 41.5, 15.8; IR (CH_2Cl_2) 3318, 1648, 1542, 1495, 1453, 1427, 1242, 1108, 739, 698 cm^{-1}; exact mass (FAB) calcd. for $C_{46}H_{47}N_2O_2Si$ (MH^+) 687.3407, found: 687.3414.

*4.2. N,N′-Bis[(2R)-hydroxy-indan-1-yl] 2-(S)-6-(S)-dibenzyl-4,4-diphenyl-4-sila-heptanediamide (**15b**)*

To a solution of 2-(*S*)-6-(*S*)-dibenzyl-4,4-diphenyl-4-sila-heptanedioic acid (529 mg, 1.04 mmol) and (1*S*,2*R*)-1-amino-2-indanol (373 mg, 2.50 mmol) in DMF (10 ml) at 0°C was consecutively added DEPC (0.38 ml, 2.50 mmol), and triethylamine (0.64 ml, 4.58 mmol). The reaction mixture was stirred at 0°C for 2 h and then warmed to room temperature overnight. The mixture was diluted with 10 ml ethyl acetate and washed successively with 5% aqueous HCl, water, saturated aqueous sodium bicarbonate, and saturated aqueous NaCl. After drying over $MgSO_4$ and filtering, the solution was concentrated in vacuo. Flash chromatography

(6:4 hexane/ethyl acetate) afforded **15b** as a foam (602 mg, 75%). $R_f = 0.25$ (6:4 hexane/ethyl acetate); mp 73–77°C; ^{1}H ($CDCl_3$) δ 7.64–6.75 (m, 28 H), 5.63 (d, 2 H, $J = 8.4$ Hz), 5.03 (dd, 2 H, $J = 4.2$ Hz and 8.1 Hz), 4.11 (m, 2 H), 2.87 (dd, 2 H, $J = 5.4$ Hz and 6.6 Hz), 2.85 (dd, 2 H, $J = 5.1$ Hz and 5.4 Hz), 2.70 (m, 4 H), 2.58 (m, 2 H), 1.88 (dd, 2 H, $J = 7.5$ Hz and 15.0 Hz), 1.63 (dd, 2 H, $J = 6.6$ Hz and 15.0 Hz), 1.3 (m, 2 H); ^{13}C ($CDCl_3$) δ 175.7, 140.2, 140.0, 139.9, 135.1, 134.8, 129.8, 129.0, 128.5, 128.2, 127.8, 126.7, 126.5, 124.9, 123.9, 72.9, 57.4, 45.5, 42.1, 38.7, 16.1; IR (KBr) 3322, 3021, 2915, 1648, 1510, 736, 700 cm^{-1}.

*4.3. N,N′-Dibenzyl 2-(S)-6-(S)-dibenzyl-4,4-dihydroxy-4-sila-heptane-diamide (**17a**)*

To a solution of diamide **15a** (166.7 mg, 0.243 mmol) in 10 ml methylene chloride at room temperature was added fresh distilled trifluoromethanesulfonic acid (0.22 ml, 2.43 mmol). After stirring at room temperature for 60 min, the mixture was cooled to 0°C and 14.8 N NH_4OH (0.25 ml, 3.64 mmol) was added. The mixture was stirred at 0°C for another 30 min then washed with water (10 ml) (aqueous layer pH = 8) and saturated aqueous NaCl (10 ml). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give silanediol **17a** (137.7 mg, 0.243 mmol, 100%); $R_f = 0.18$ (5:1 benzene/acetone); ^{1}H NMR (DMSO-d_6) δ 8.02 (t, 2 H, $J = 5.4$ Hz), 7.23–6.81 (m, 20 H), 4.31 (dd, 2 H, $J = 15$ Hz and 6.6 Hz), 3.90 (dd, 2 H, $J = 15$ Hz and 4.8 Hz), 2.81–2.79 (m, 6 H), 0.93–0.89 (m, 4 H); ^{13}C NMR (DMSO-d_6) δ 175.8, 175.6, 175.5, 140.7, 140.5, 140.4, 139.5, 139.4, 139.3, 129.1, 129.0, 128.2, 127.1, 127.0, 126.6, 125.9, 42.9, 42.8, 42.7, 41.8, 40.5, 40.2, 20.4, 19.9, 19.8; IR (KBr) 3396, 1647, 1541, 1496, 1455, 1384, 1246, 1076, 891, 741, 698 cm^{-1}.

*4.4. N,N′-Bis[(2R)-hydroxy-indan-1-yl] 2-(S)-6-(S)-dibenzyl-4,4-dihydroxy-4-sila-heptanediamide (**17b**)*

To a solution of diamide **15b** (62 mg, 0.08 mmol) in methylene chloride (3 ml) at room temperature was added freshly distilled trifluoromethanesulfonic acid (0.14 ml, 1.6 mmol). After stirring at room temperature for 40 min, the mixture was cooled to 0°C and 14.8 N NH_4OH (0.16 ml, 2.4 mmol) was added. The mixture was stirred at 0°C for another 30 min then filtered and the solid was washed with methylene chloride (2 ml) followed by water (5 ml). Drying under the vacuum overnight provided silanediol **17b** (19.2 mg, 0.03 mmol, 37%); ^{1}H NMR (DMSO-d_6) δ 7.61 (d, 2 H, $J = 8.6$ Hz), 7.27–6.95 (m, 18 H), 6.05 (br, 2 H), 5.04 (dd, 2 H, $J = 4.8$ Hz and 12.8 Hz), 4.60 (d, 2 H, $J = 3.5$ Hz), 4.22 (m, 2 H), 2.90–2.66 (m, 10 H), 0.91 (dd, 2 H, $J = 6.5$ Hz and 15.1 Hz), 0.70 (dd, 2 H, $J = 6.5$ Hz and 14.5 Hz); ^{13}C NMR (DMSO-d_6) δ 176.1, 142.2, 140.7, 140.6, 129.0, 128.1, 127.0, 126.0, 125.8, 124.7, 124.2, 72.2, 56.7, 42.5, 40.2, 39.7; IR (KBr) 1638, 1618, 1526, 1400, 1384, 1259, 1178, 1036, 982, 886, 750, 704, 648 cm^{-1}.

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