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(Trimethylsilyl)alanine: a Metabolically Stable 'Bio-Isostere' for Phenylalanine

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Abstract. The preparation of protected derivatives of enantiomerically pure β-(trimethylsilyl)alanine (**1**) and their chemical properties are decribed. Examples of renin inhibitory peptides, containing **1** are given, which demonstrates its use as a 'bio-isosteric' replacement for phenylalanine (**2**).

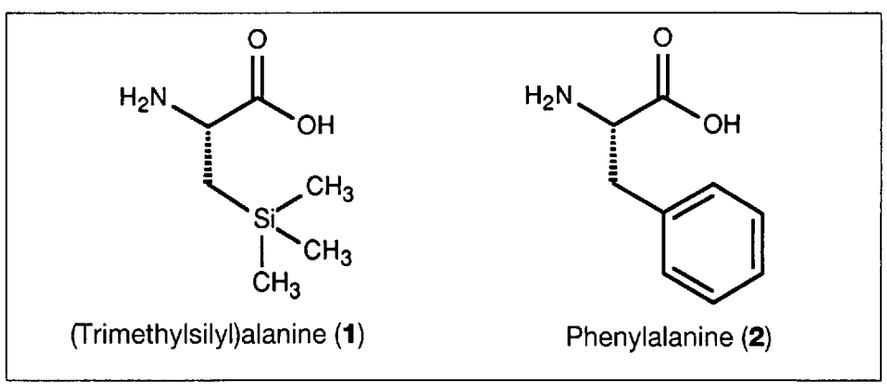
Introduction

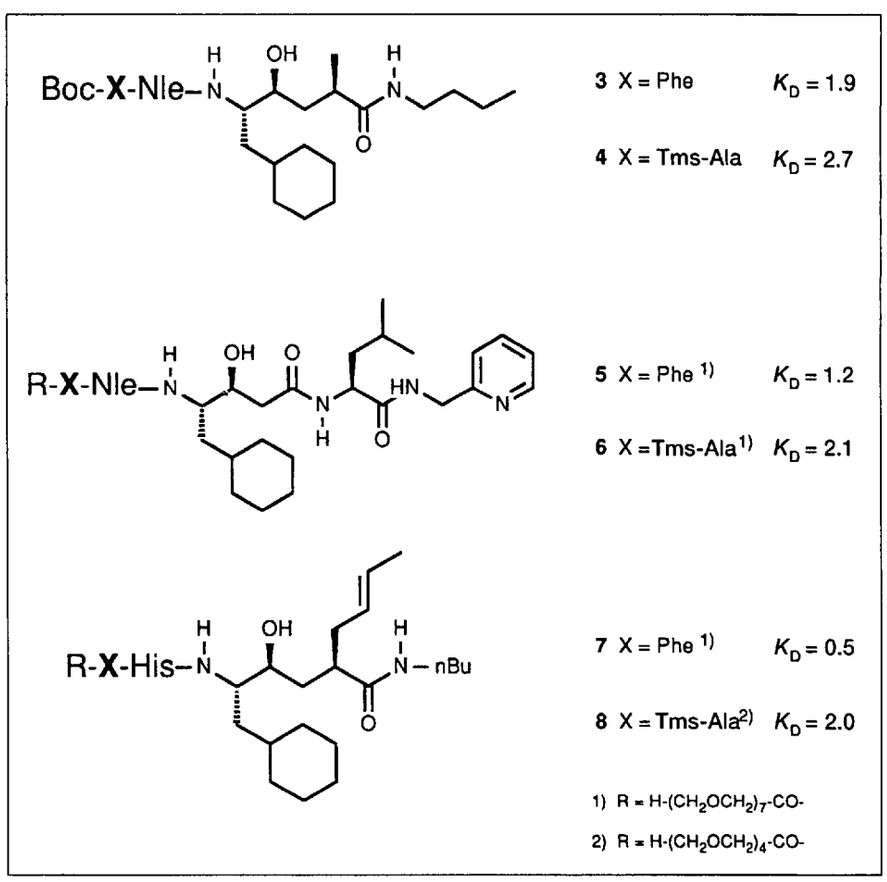
Over the last decades, peptides have became one of the most popular tools for the medicinal chemists. In medical therapy too, peptides play an increasingly important role, although only a few of them have become important therapeutics. Examples are insuline, calcitonin and, most recently *Sandostatin*®, a somatostatin analog. In a broader sense, also antibodies, enzymes, and factors such as cytokines belong to this class of drugs. Crucial for this development was the discovery and sequencing of a large number of endogenous, regulatory peptides over the last 40 years [1]. Biotechnology and the availability of modern *synthetic* methods, especially automated solid phase synthesis, enabled the efficient assembly of the peptides. Recently, peptides have also been proposed for the random generation of large number of compounds, so-called peptide libraries, using chemical synthesis or biotechnology to produce them [2].

The other side of the coin is that peptides identified as 'lead' compounds are seldom ideal drug candidates: they are inactive upon oral administration and, when given parentally, quickly degraded and/or eliminated. Especially 'at risk' are the amide bonds of phenylalanine, tyrosine, arginine, and lysine. One of the many possibilities to stabilize these bonds is to use synthetic amino acids with 'unnatural' side chains, which are no longer substrates for proteolytic enzymes. In our studies for stable renin inhibitors we investigated β-(trimethylsilyl)alanine (**1**, Tms-Ala) [3], as a *bioisosteric* [4] replacement for phenylalanine (**2**).

Results and Discussion

Starting with *Schöllkopf*'s reagent (**9**) [5], Tms-Ala (**1**) is easily prepared in *both* enantiomeric forms according to the *Scheme*. It is resistant against most of the reagents used in peptide chemistry, such as Pd-C/H_2, CF_3COOH/CH_2Cl_2, piperidine or Et_3N in DMF, NaOH in CH_3OH or H_2O, conc. NH_3, and $Bu_4N^+F^-$ in THF. Furthermore, the conditions used for peptide coupling like DCC/HOBT [6] or *Cas-*





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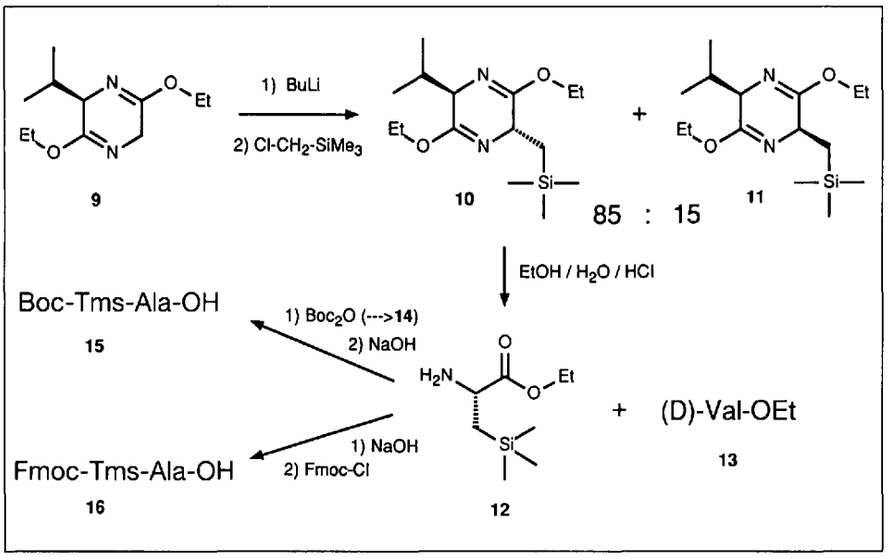
Scheme



tro's reagent [7] leave its side chain untouched. Only stronger acids like HCl in AcOH cleave the Tms-Ala peptides slowly to the corresponding alanine derivatives.

As is evident from the renin inhibitors [8] **3** to **8** β-(trimethylsilyl)alanine (**1**) appears to be an excellent replacement for phenylalanine (**2**). There is only a marginal loss in affinity to the enzyme measured as a small increase of the K_D values [9]. On the other hand, all the peptides containing **1** are resistant towards proteolytic digestion with α-chymotrypsin, in sharp contrast to the corresponding **2** containing peptides. As an example, **7** is quantitatively degraded by α-chymotrypsin within 30 min (phosphate buffer pH 7, 1 mg α-chymotrypsin/ml), whereas the Tms-Ala derivative **8** remains unchanged under the same conditions for many hours. In addition, replacement of **2** by **1** means that there is no longer an aromatic ring susceptible to metabolic hydroxylation, a transformation often observed *in vivo*. Last but not least, the higher lipophilicity of the Tms-ala peptides might also be an advantage.

Incorporation of β-(trimethylsilyl)alanine (**1**) in place of phenylalanine (**2**) (or leucine) in other biologically active peptides will show the scope and limitation of these new 'bio-isosterism' described here.

Experimental Part

General. The N- and/or C-terminal protected intemediates were synthesized as described below. Incorporation of these building blocks into compounds **3**–**8** was performed using standard procedures for peptide synthesis [10]. The preparation of the 'transitionstate' analogs within structures **3**–**8** have been described in [8][11].

(R)-β-(Trimethylsilyl)alanine Ethyl Ester (**10**). To a soln. of 24 g of *(2R)-2,5-dihydro-3,6-diethoxy-2-isopropylpyrazine* (**9**) in 400 ml of THF were slowly added 70 ml of BuLi (1.6M in hexane) at –70°. After stirring for additional 15 min, 30 g of (chloromethyl)trimethylsilan were added and the mixture was allowed to reach r.t. overnight. It was diluted with Et_2O, washed with H_2O, dried ($MgSO_4$), and the solvent evaporated. The residue was purified chromatographically using 600 g of silica gel and 1% Et_2O in hexane as eluant. The *(R,R)*-isomer **10**, which eluted first, was dissolved in 200 ml of EtOH, cooled to 0–5°, treated with 70 ml of 10% HCl and kept at this temp. for 2 h. After evaporation, the residue was distributed between CH_2Cl_2 and 2N Na_2CO_3. The org. layer was carefully concentrated on a rotary evaporator at r.t. and chromatographed using hexane/Et_2O 4:1 that had been made alkaline by shaking with 50 ml of conc. NH_3/l solvent. The first eluted **12** could be stored for several weeks in a freezer (–25°). Yield 13 g (60%). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.02 (*s*, 9 H); 0.8 (*dd*, 9, 15, 1 H); 1.0 (*dd*, 7, 15, 1 H); 1.2 (*t*, 7, 3 H); 1.4 (br. *s*, 2 H); 3.4 (*dd*, 7, 9, 1 H); 4.1 (*m*, 2 H). A sample was converted to its toluene sulfonate: colorless crystal M.p. 119–120°; $[\alpha]_D$ = +17.6 (*c* = 2.0 in EtOH). Anal. calc. for $C_{15}H27NO_5SiS$ (361.5): C 49.86, H 7.47, N 3.87; found: C 49.9, H 7.6, N 4.0.

(R)-N-(tert-Butoxycarbonyl)-β-(trimethylsilyl)alanine Ethyl Ester (**14**). To a soln. of 5 g of **12** in THF were added 6 g of Boc-anhydrid. The mixture was stirred overnight and then evaporated to dryness at 50°. Yield 7.5 g (82%) of colorless oil, which solidified on standing to a low-melting wax-like solid. $[\alpha]_D$ = +3.5 (*c* = 1.0 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.04 (*s*, 9 H); 0.9 (*dd*, 9,15, 1 H); 1.1 (*dd*, 7, 15, 1 H); 1.25 (*t*, 7, 3 H); 1.4 (*s*, 9 H); 4.15 (*m*, 2 H); 4.25 (*m*, 1 H); 4.85 (br. *s*, 1 H). Anal. calc. for $Cl_3H_{27}NO_4Si$ (289.5): C 53.98, H 9.34, N 4.84; found: C 54.0, H 9.3, N 4.8.

(R)-N-(tert-Butoxycarbonyl)-β-(trimethylsilyl)alanine (**15**). A soln. of 7 g of **14** in MeOH/H_2O 4:1, cooled to 0–5° was slowly treated with a soln. of 1.5 g of NaOH in H_2O. After 3 h MeOH was evaporated, the residue acidified with orthophosphoric acid and extracted with Et_2O. Yield 6.1g (97%) of colorless solid, m.p. 109–111°; $[\alpha]_D$ = –7.6 (*c* = 0.5 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.02 (*s*, 9 H); 0.9 (*dd*, 11, 15, 1 H); 1.1 (*dd*, 6, 15, 1 H); 1.4 (*s*, 9 H); 4.25 (br. *m*, 1 H); 4.8 (br. *m*, 1 H). Anal. calc. for $C_{11}H_{23}NO_4Si$ (261.4): C 50.58, H 8.81, N 5.36; found: C 50.5, H 8.7, N 5.3.

(R)-N-[(Fluoren-9-yl)methoxycarbonyl]-β-(trimethylsilyl)alanine (**16**). A soln. of 4.8 g of **12** in MeOH/H_2O 4:1, was cooled to 0–5° and hydrolyzed by adding a soln. of 1.1 g of aq. NaOH. After 6 h, 100 ml of H_2O, 2.3 g of $NaHCO_3$ and 3.8 g of Na_2CO_3 were added. Most of the MeOH was evaporated, the residue (*ca.* 80 ml of soln.) diluted with 80 ml of dioxane and cooled to 0–5°. 6.56 g of Fmoc-chloride was added in small portions. After stirring at r.t. overnight, the mixture was diluted with 1 l H_2O and 50 ml of conc. NaCl. The aq. phase was extracted with Et_2O, acidified with conc. HCl and again extracted with Et_2O. The second extract was dried ($MgSO_4$) and the solvent evaporated. The residue was chromatographed with CH_2Cl_2/CH_3OH/AcOH 100:2:0.5 as eluant. Yield 8 g (82%) of amorphous solid, $[\alpha]_D$ = –9.1 (*c* = 1.0 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.02 (*s*, 9 H); 0.95 (*dd*, 11,15,1 H); 1.15 (*dd*, 6, 15, 1 H); 4.15 (*t*, 7, 1 H); 4.35 (*m*, 3 H); 5 (br. *d*, 8, 1 H); 7.2–7.8 (*m*, 8 H). Anal. calc. for $C_{21}H_{25}NO_4Si$ (383.5): C 65.80, H 6.52, N 3.65; found: C 65.6, H 6.6, N 3.7.

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