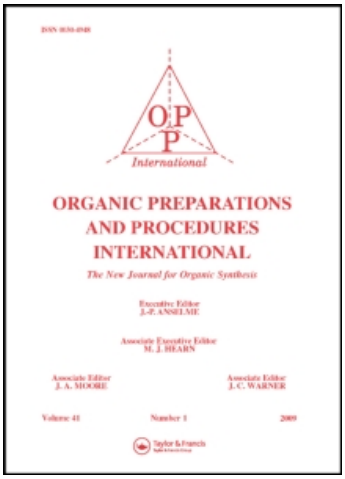

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PREPARATIVE SYNTHESIS OF RL-252 VANADYL COMPLEX

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PREPARATIVE SYNTHESIS OF RL-252 VANADYL COMPLEX

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The insulinomimetic property of vanadyl (VO^{2+}) and vanadate (VO_3^{1-}) ions has been the topic of considerable research since 1980.[1-6] Recently, this work has been extended by a number of laboratories to include the use of VO^{2+} that is complexed to various hydrophobic organic chelating agents.[7-9] These hydrophobic complexes, in principle, offer the promise of increasing the oral availability of vanadyl cations and thus have potential as insulin substitutes for the treatment of diabetes. In order to investigate this potential utility, we set out to synthesize a number of the reported VO^{2+} complexes. One such complex is reported with an organic chelating agent named RL-252 (**6**) but the complex was only prepared *in situ*.[7] To date, there has been no report of a method for preparation and isolation of the RL-252 vanadyl complex (**8**) in gram quantities. We now describe such a method.

Our synthetic approach was based on that reported by Schechter, *et al.*[7] We found a number of the steps to be difficult on a large scale and thus have altered the synthesis, changing both protecting group strategies and synthetic methods. Additionally, the reported synthesis gave little detail to the spectral characterization of synthetic intermediates, which are reported for our modified synthesis herein.

A hydroxide-catalysed Michael addition of commercially available 2,2-dimethyl-1,3-dihydroxypropane to acrylonitrile gave **1** and subsequent acid hydrolysis afforded **2**. Initially, we found the nitrile hydrolysis difficult to reproduce but monitoring the progress of the reaction by a combination of TLC (to detect disappearance of **1**) and IR spectroscopy (to detect complete hydrolysis of the intermediate amides) alleviated this difficulty. Prolonged hydrolysis gave substantial impurities resulting from β-elimination side reactions.

For the synthesis of leucine N-methylhydroxamate (**5**), N-Boc-L-leucine was converted to a mixed anhydride and treated *in situ* with O-trimethylsilyl-N-methylhydroxylamine, also prepared *in situ* according to the method of Nakonieczna and Chimiak.[10] This method uses the temporary protection by the TMS group to avoid competition between O- and N-acylation. The TMS group was cleaved in the work-up, affording **4** as a stable, crystalline solid in good yield. In some cases, **4** crystallized

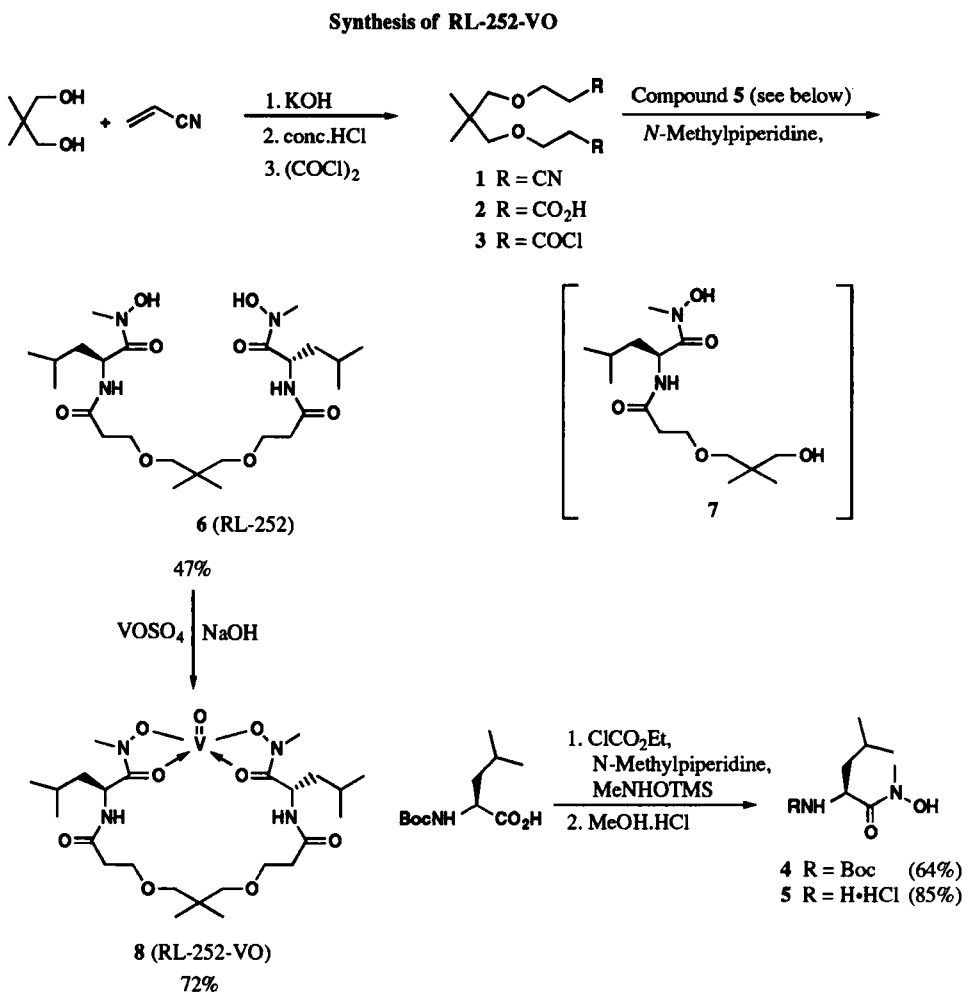

Fig. 1

directly from the reaction mixture in 30-40% yield but typically, higher yields were achieved using the filtration through silica gel described in the Experimental Section. It is worth noting that the NMR spectrum of **4** in $CDCl_3$ shows an E/Z mixture of hydroxamate tautomers. This material displayed a single spot by TLC whereas reactions that produce mixtures of O- and N-acylation show additional resonances attributable to the O-acylation product in the NMR spectrum and two spots by TLC. Removal of the Boc protecting group is accomplished by treatment with methanolic hydrogen chloride to give **5** in over 80% yield as a crystalline hydrochloride which was conveniently converted to its free base by treatment with anhydrous ammonia in chloroform.

The activation of **2** and subsequent reaction with **5** proved to be the two most difficult reactions of the synthesis. Numerous methods for this coupling were investigated: *a*) reaction of **5** with mixed anhydrides of **2**, *b*) use of carbodiimide coupling reagents, *c*) preparation and isolation of acti-

vated ester of **2** followed by reaction with **5**, and *d*) acylation of **5** with the *bis*-acid chloride (**3**). All of these methods suffer from β-elimination side-reactions, giving rise to impurities such as **7** that are extremely difficult to separate from **6**. The *bis*-acid chloride method gave the best yield since the activation of **2** is essentialy quantitative. All other means of carboxylate activation showed evidence of β-elimination prior to the addition of **5**. β-Elimination, though still a problem in the reaction of **3** with **5**, was minimized by the use of N-methylmorpholine as a proton scavenger rather than the tradititional, more basic trialkylamines. Repeated chromatography was necessary to obtain pure **6**. This required the use of iron-free stationary phase and solvents since **6** avidly chelates Fe^{2+} and Fe^{3+} ions. The presence of the RL-252 iron complexes was easily detected by a rust-like color whereas pure **6** is essentially colorless. Iron-contaminated **6** was easily purified by chromatography on iron free silica gel since the iron complexes elute more slowly than **6**. For the combination of carboxylate activation and amide forming reactions, the method described herein gave roughly a three-fold enhancement of yield compared to the previously described method.[7]

Treatment of an ethanolic solution of **6** with aqueous vanadyl sulfate followed by rapid adjustment to pH 5 with aqueous NaOH afforded **8** as a purple solid. The presence of catalytic amounts of sodium ascorbate[11] as an oxygen scavenger dramatically enhanced the yield and purity of **8** by preserving the vanadyl oxidation state. Although mass spectral data and elemental analyses are consistent with the desired cyclic complex, the possible inclusion of cyclic and linear polymeric complexes cannot totally be dismissed. So far we have not found a method for growing crystals suitable for x-ray crystallography.

The overall synthesis of RL-252 vanadyl complex (**8**) requires seven steps and the longest linear sequence of five steps is accomplished in approximately 30% yield. We believe the route described is viable for preparation of gram quantities of this insulinomimetic agent.

EXPERIMENTAL SECTION

Reagent grade solvents were used as supplied from commercial sources. Acrylonitrile, 1,3-dihydroxy-2,2-dimethylpropane, oxalyl chloride, TMS-Cl, N-methyl-hydroxylamine hydrochloride, ethyl chloroformate and vanadyl sulfate were purchased from Aldrich Chemical Company. Sodium Ascorbate was purchased from Sigma Chemical Company. BOC-L-leucine was purchased from Bachem, Inc. Pyridine, N-methylmorpholine and N-methylpiperidine were distilled from CaH_2. Melting points were obtained using a Thomas Hoover capillary melting point apparatus. NMR spectra were obtained using one of the following spectrometers and resonances are reported in ppm downfield from TMS: Varian XL 300, Gemini 200, Unity 400 or Bruker AM 250. IR spectra were obtained using either Mattson Sygnus 100 or Biorad FTS 45 spectrometers and absorbances reported in cm^{-1}. Mass spectra were obtained using one of the following spectrometers and data are reported as mass/charge ratio in atomic mass units: Finnigan 4500, TSQ-70, MAT900; VG Analytical 7070E/HF, Masslab Trio-2A; or Fisons VG Trio 2000. C, H and N analyses were performed using a Leeman Lab model 440 elemental analyser.[12] Vanadium analyses were performed by E & R Microanalytical Laboratory.[13] All preparative chromatographic separations were performed using "low iron" silica gel (Aldrich Chemical Co. catalog #28,851-9, Fe content ≤10 ppm). The silica gel from the initial purification of RL-252 was recovered, washed with methanol, dried at ca. 15 mmHg, 100° and used in subsequent chromato-

graphic purifications to ensure an iron free stationary phase. All reactions were carried out under nitrogen atmosphere.

1,3-Bis(2-cyanoethoxy)-2,2-dimethylpropane (1).- A mixture of acrylonitrile (13.2 mL, 201 mMol) and 1,3-dihydroxy-2,2-dimethylpropane (9.4 g, 90.3 mMol) was treated with 50% aqueous KOH (0.48 mL, 6 mMol) and stirred at RT for 18 hrs. The reaction mixture was cooled on an ice bath, neutralized with 0.5 M HCl_{aq}, and diluted with EtOAc (300 mL). The resulting organic solution was washed with water and saturated aqueous NaCl, dried over $MgSO_4$, filtered and evaporated to give **1** (18.3 g, 96%) as a pale yellow liquid. ^1H NMR ($CDCl_3$): δ 3.65 (t, 4H); 3.25 (s, 4H); 2.57 (t, 4H); 0.91 (s, 6H). IR ($CHCl_3$): 2872; 2255. MS (CI, 1% NH_3 in CH_4): 211 (m + 1); 140 (base, m-C_3H_4NO).

Anal. Calcd for $C_{11}H_{18}N_2O_2$: C, 62.83; H, 8.63; N, 13.32. Found: C, 62.68; H, 8.42; N, 13.25

3,3'-[(2,2-dimethyl-1,3-propanediyl)bis(oxy)]bis[propanoic Acid] (2).- A mixture of **1** (30 g, 143 mmol) and conc. HCl (35 mL) were stirred at 95° for 4.5 hrs. The reaction mixture was cooled, poured into ice water (80 mL) and the product was extracted with a 1:1 mixture of Et_2O and EtOAc (2 x 100 mL). The combined organic layers were washed with saturated, aqueous NaCl, dried over $MgSO_4$, filtered and concentrated at reduced pressure to afford **2** (35 g, 99%) as a pale yellow oil. ^1H NMR ($CDCl_3$): δ 10.25 (br s, 2H); 3.68 (t, 4H); 3.18 (s, 4H); 2.57 (t, 4H), 0.82 (s, 6H). IR (film on NaCl): 2950, 1730. MS (CI, 1% NH_3 in CH_4): 249 (m + 1); 159 (m-$C_3H_5O_3$); 141(base, 159 - H_2O).

Anal. Calcd for $C_{11}H_{20}O_6$•0.15 H_2O: C, 52.64; H, 8.15; H_2O, 1.08. Found: C, 52.73; H, 8.03; H_2O, 1.25

(S)-2-[[(1,1-Dimethylethoxy)carbonyl]amino]-N-hydroxy-N,4-dimethylpentanamide (4).- A solution of N-methylhydroxylamine•HCl (14 g, 143.6 mMol) in dry pyridine (150 mL) was chilled on an ice bath and treated dropwise with a solution of TMS-Cl (38.0 mL, 300 mmol) in CH_2Cl_2 (100 mL) and stirred for 1.25 hr (Solution A). A solution of N-BOC-L-leucine hydrate (25 g, 100 mMol) was dried over $MgSO_4$ and the solids were removed by filtration, rinsing with additional CH_2Cl_2 (100 mL). The combined filtrate and washings were chilled to -10° and treated with N-methylpiperidine (13.0 mL, 107 mMol) followed by ethyl chloroformate (10.0 mL, 105 mMol). After 2 min the resulting solution was transferred via cannula to Solution A above. The reaction mixture was allowed to warm gradually to RT overnight and then it was concentrated to a thick slurry at reduced pressure. The slurry was partitioned between Et_2O and 0.5 N HCl. The organic layer was washed repeatedly with 0.5 N HCl until the aqueous layer remains acidic. The Et_2O layer was then washed with saturated NaCl, dried over $MgSO_4$, filtered and evaporated. The residue was filtered through a bed of silica gel, eluting with a gradient of $CHCl_3$ to $CHCl_3$-MeOH (97:3) to give a partial purification of the major product. The product fractions were evaporated, dissolved in hexanes and evaporated again. The residue was taken up in hexanes and chilled on an ice bath to give crystalline **4**. The liquor was diluted with CH_2Cl_2 and washed consecutively with 0.5N HCl, 5% $NaHCO_3$ and saturated NaCl. It was then diluted with hexanes, dried over $MgSO_4$ and concentrated to afford a second crop. Similar treatment of the liquor gave a third crop. All three crops combined (17.2 g) were dissolved in CH_2Cl_2, diluted

with hexanes and concentrated at reduced pressure until a thick slurry resulted. This slurry was chilled on an ice bath, diluted with hexanes and filtered. The resulting solid was dried to constant weight at reduced pressure to afford **4** (16.1 g, 64%) as a colorless solid. mp. 110-111°. ^{1}H NMR ($CDCl_3$): δ 9.35 (br, 1H); 5.19 and 5.12 (d, 0.8 and 0.2H); 4.81 and 4.53 (q, 0.8 and 0.2H); 3.46 and 3.24 (s, 0.6 and 2.4H); 1.67 (m, 2H); 1.47 (m, 1H); 1.42 (s, 9H); 0.93 (d, 6H). IR (KBr): 3340, 3310, 1700, 1680, 1625, 1530. MS (CI, 1% NH_3 in CH_4): 261 (m + 1); 205 (base, m-C_4H_8); 161 (m-Boc + 1). $[\alpha]_D^{23}$ -4.7° (0.84%, MeOH).

Anal. Calcd for $C_{12}H_{24}N_2O_4$: C, 55.36; H, 9.29; N, 10.76. Found: C. 55.20; H, 9.23; N, 10.54

(S)-2-Amino-N-hydroxy-N,4-dimethylpentanamide Monohydrochloride (5).- A solution of **4** (9.86 g, 37.9 mMol) in CH_2Cl_2 (75 mL) was treated with freshly prepared methanolic hydrogen chloride (75 mL) and stirred at RT for 90 min. The resulting solution was evaporated and the residue was dissolved in dry THF and evaporated to a wet solid that was triturated with dry THF and collected by filtration. Drying at ca 15 mmHg, 25° overnight gave **5** (6.36 g, 83%) as a colorless solid. mp. 151-153°. ^{1}H NMR (D_2O): δ 4.83 (s, 5H, HOD); 4.57 (t, 1H); 3.28 (s, 3H); 1.72 (m, 3H); 0.97 (d, 6H). IR (KBr): 3420, 2950, 1645, 1620, 1530. MS (DEI): 161 (m + 1); 160 (m); 86 (base, m-CON(Me)OMe). $[\alpha]_D^{23}$ +4.3° (1.07%, MeOH).

Anal. Calcd for $C_7H_{16}N_2O_2$•HCl: C, 42.75; H, 8.71; N, 14.24; Cl, 18.03
Found: C, 42.70; H, 8.59; N, 14.12; Cl, 17.62

(2S,16S)-N,N'-Dihydroxy-N,N',9,9-tetramethyl-2,16-bis(2methylpropyl)-4,14-dioxo-7,11-dioxa-3,15-diazaheptadecanediamide (RL-252 (6).- To a 0° solution of **2** (6.10 g, 24.4 mMol) in CH_2Cl_2 (20 mL) and DMF (0.1 mL) was added oxalyl chloride (10.8 g, 85.4 mMol), dropwise over 30 min. Upon completion of the addition, the mixture was allowed to warm to RT and was stirred 60 min. The solution was concentrated at reduced pressure, dissolved in CH_2Cl_2 and evaporated again to afford the crude diacid chloride, **3** (9.0 g, 96%). ^{1}H NMR ($CDCl_3$): δ 3.69 (t, 4H); 3.19 (s, 4H); 3.10 (t, 4H); 0.84 (s, 6H). This material was used without further purification or characterization below.

Ammonia gas was bubbled through a suspension of **5** (10.3 g, 52.4 mMol) in $CHCl_3$ (15 mL) while cooling with an ice bath for 10 min. The resulting mixture was allowed to stand an additional 10 min before filtering off NH_4Cl. The filtrate was evaporated at reduced pressure to give the free base of **5** (8.4 g, 100%) as a colorless oil. NMR ($CDCl_3$): δ 8.30 (br, 1H); 4.28 (m, 1H); 3.17 (s, 3H); 1.89-1.45 (m, 3H); 0.89 (d, 6H). This material was used without further purification or characterization below.

Free base **5** and N-methyl morpholine (5.3 g, 52.4 mMol) were dissolved in CH_2Cl_2 (20 mL) and added dropwise over 15 min to a 0° solution of crude diacid chloride, **3**, in CH_2Cl_2 (20 mL). After the addition was complete, the mixture was stirred for 15 min before diluting with ether (100 mL) and washing with 2N $HCl_{(aq)}$ and saturated $NaCl_{(aq)}$. The organic layer was dried over $MgSO_4$ and evaporated to a gum (11 g). Silica gel (200 g) was slurried in $CHCl_3$ and packed into a 6 cm diameter column. The crude material was dissolved in $CHCl_3$ (20 mL) and loaded onto the column. The column was eluted with a gradient of $CHCl_3$ to $CHCl_3$-MeOH (97:3), collecting 50 mL fractions.

Purity of the factions were monitored by TLC (Silica gel, $CHCl_3$-MeOH, 9:1). Head fractions of the major component, containing significant amounts of close R_f impurities by TLC were separated and the purest fractions were combined and evaporated to give a gum. Further drying at 0.5 mmHg, RT, overnight gave **6** (7.2 g) as a crisp, pale yellow foam. This material was rechromatographed on recycled silica gel (see General section above), separating impure head fractions as above to give **6** (6.1 g, 47% from **2**) as a colorless foam upon drying to constant weight at 0.5 mmHg, RT. ^1H NMR ($CDCl_3$ + D_2O): δ 5.14 (m, 2H); 3.63 (m, 4H); 3.24 (s, 6H + d, 2H); 2.94 (d, 2H); 2.42 (m, 4H); 1.66 (m, 4H); 1.50 (m. 2H); 0.95 (m, 15H); 0.83 (s, 3H). IR (KBr): 3435, 3285, 1632, 1111, 698. MS (CI, 1% NH_3 in CH_4): 533 (m + 1); 486 (m-MeNOH + 1); 485 (m-MeNOH).

$[\alpha]_D^{23}$ -5.7° (0.91%, $CHCl_3$).

Anal. Calcd for $C_{25}H_{48}N_4O_8 \cdot 0.05CHCl_3$: C, 55.86; H, 8.99; N, 10.40; Cl, 0.99
Found: C, 56.08; H, 8.85; N, 10.01; Cl, 0.57

Vanadyl complex with (2S, 16S)-N,N'-Dihydroxy-N,N',9,9-tetramethyl-2,16-bis(2-methylpropyl)-4,14-dioxo-7,11-dioxa-3,15-diazaheptadecanediamide (RL-252-VO (8).- A solution of **6** (3.06 g, 5.75 mMol) in EtOH (30 mL) was purged with N_2 and treated with sodium ascorbate (25 mg, 0.13 mMol). The resulting mixture was treated dropwise over 5 min with a solution of $VOSO_4$ in N_2 purged water. The resulting purple solution was immediately adjusted to pH 5 by dropwise addition of 1N NaOH, leading to the precipitation of **8** as a purple solid. Water (30 mL) was added dropwise and the resulting suspension was chilled on an ice bath for 15 min. Filtration of the solid followed by rinsing with cold water and then with cold ethanol gave **8** which was dried at ca. 15 mmHg, RT for 48 hrs to yield 2.48 g (72.2%), mp. 194-195° (decomposes). MS (FAB, thioglycerol): 598 (m + 1); 582 (m-CH_3); 439 (base, m-$VO(ONMe)_2$ + 1). IR (KBr): 3435, 1632, 1605, 1107, 991, 698, 592.

Anal. Calcd for $C_{25}H_{46}N_4O_9V \cdot H_2O \cdot C_2H_6O$: C, 49.01; H, 8.22; N, 8.46; V, 7.70
Found: C, 48.87; H, 7.87; N, 8.44; V, 8.06

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