Title: Non-peptidyl small molecule, adenosine, 5'-Se-methyl-5'-seleno-, 2',3'-diacetate, activates insulin receptor and attenuates hyperglycemia in type 2 diabetic *Lepr*$^{db/db}$ mice

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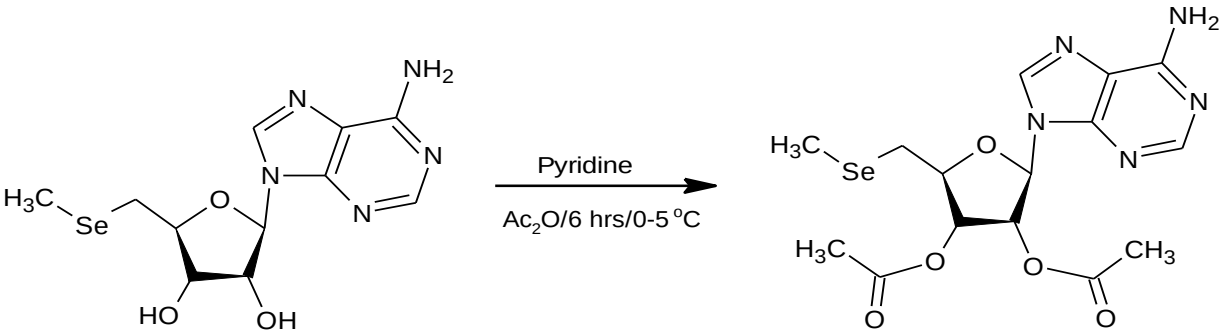
Supplementary Material-1

Synthesis of NPC43 (Adenosine, 5'-*Se*-methyl-5'-seleno-, 2',3'-diacetate)

Scheme:

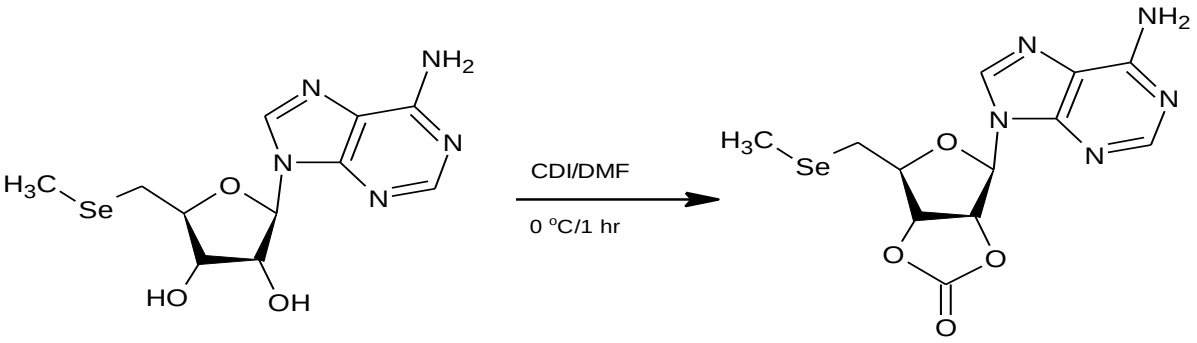


Synthetic procedure: 5'-*Se*-methyl-5'-seleno-Adenosine (1.0 gr, 0.0029 mole, 1.0 mole eq.) and anhydrous pyridine (10 ml) were placed in an oven dried, 50 ml three neck flask, equipped with a dropping funnel, inert gas inlet/outlet and a thermometer. The reaction set was placed in an ice/salt bath and agitation was initiated. When the temperature of the solution dropped to 0^{o}C, acetic anhydride (10 ml, 0.105 mole, 36.47 mole eq.) was added drop-wise for 15 minutes and the temperature of the reaction mixture was maintained below 5^{o}C during acetic anhydride addition. The reaction mixture was stirred for 6 hours at 0-5^{o}C. The excess acetic anhydride was quenched by adding ice-cold water (100 ml), and then pH adjusted to 7 by adding 10% (wt/vol) $NaHCO_3$ aqueous solution. The aqueous mixture was extracted with ethyl acetate (2 x

100 ml). The combined ethyl acetate extracts were dried over anhydrous Na_2SO_4 (1gr) and filtered into a 250 ml round-bottomed flask. The filtrate was concentrated to dryness under reduced pressure at 35-40^oC to yield a crude product as a pale yellow syrupy liquid. Pure product was then obtained as off-white crystals (1.12 gr, Yield: 90.3 %, Purity by HPLC and mass spectrometry: >99%) by passing the yellow liquid through a silica gel column with a mixture of ethyl acetate and hexanes (1:3 v/v).

Synthesis of Adenosine, 5'-*Se*-methyl-5'-seleno-, cyclic 2',3'-carbonate (Compound #50):

Scheme:

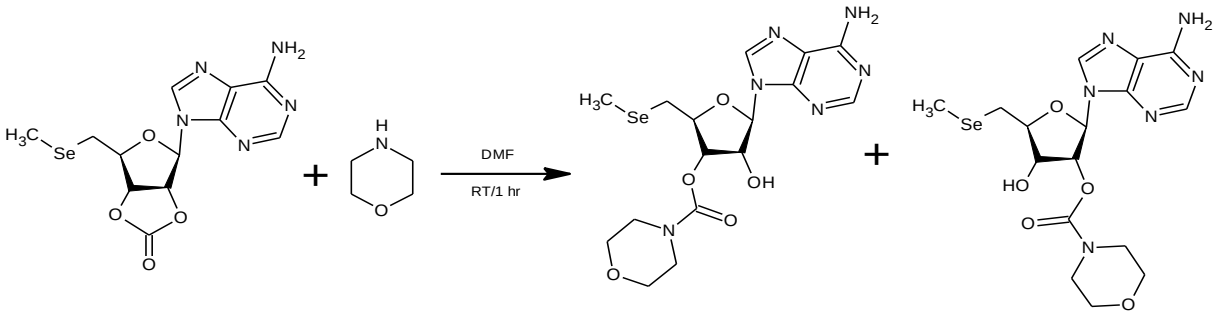


Synthetic procedure: 5'-*Se*-methyl-5'-seleno-Adenosine (1.0 gr, 0.0029 mole, 1.0 mole eq.) and anhydrous dimethylformamide (20 ml) were placed in an oven dried, 50 ml three neck flask, equipped with a dropping funnel, inert gas inlet/outlet and a thermometer. The reaction set was placed in an ice/salt bath and agitation was initiated. When the temperature of the solution dropped to 0^oC, carbonyldiimidazole (CDI, 0.57 gr, 0.0035 mole, 1.21 mole eq.) was added at below 5^oC. The reaction mixture was slowly warmed to the room temperature, and then stirred the reaction mixture for 4 hours at the same temperature under argon gas atmosphere. The solvent was removed under reduced pressure to yield a residue, which was then dissolved in a mixture of chloroform (5 ml) and ethanol (few drops) to get clear solution. The organic layer was washed with 1% aq. acetic acid solution (2 x 1 ml) and dried over anhydrous Na_2SO_4 (1 gr), filtered into a 250 ml round-bottomed flask. The filtrate was concentrated to dryness under reduced pressure at 25-30^oC to yield a crude product as a pale yellow syrupy liquid. The crude product was dissolved in a mixture of ethanol/water mixture (1:1 v/v), and then concentrated to dryness under reduced pressure at 45-50^oC to give a residue to which hexane (25 ml) was added

and stirred for 10 minutes. The mixture was concentrated to dryness under reduced pressure at 30-35^{o}C to yield the desired product as a off-white crystals (1.02 gr, Yield: 95.3 %, Purity by HPLC and mass spectrometry: >99%).

Synthesis of a regio-isomeric mixture of Adenosine, 5'-*Se*-methyl-5'-seleno-, 2'-(4-morpholinocarbamate) and Adenosine, 5'-*Se*-methyl-5'-seleno-, 3'-(4-morpholinocarbamate) (Compound #53):

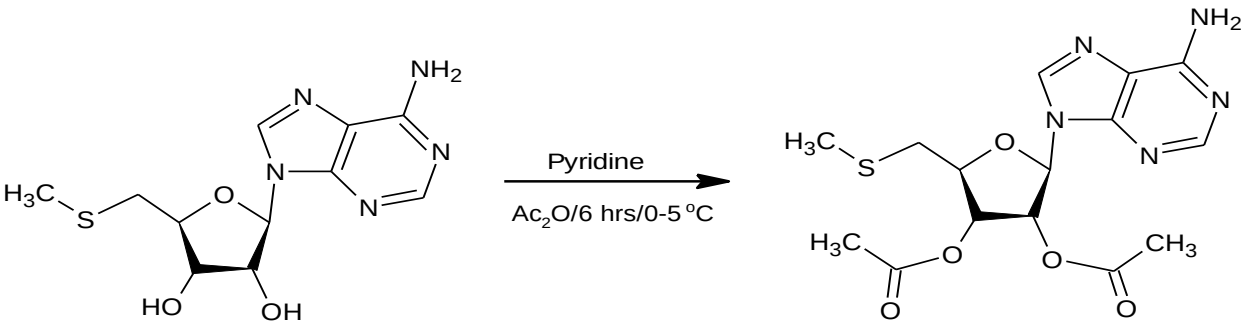
Scheme:



Synthetic procedure: Adenosine, 5'-*Se*-methyl-5'-seleno-, cyclic 2',3'-carbonate (1.0 gr, 0.0027 mole, 1.0 mole eq.) and anhydrous dimethylformamide (10 ml) were placed in an oven dried, 50 ml three neck flask, equipped with a dropping funnel, inert gas inlet/outlet and a thermometer. Morpholine (0.26 gr, 0.0029 mole, 1.1 mole eq.) was added at 20-25^{o}C. The reaction mixture was stirred for 1 hour at room temperature, and then concentrated to dryness under reduced pressure at 45-50^{o}C to yield a residue. Hexane (25 ml) was added and stirred for 10 minutes to precipitate the desired regio-isomeric mixture product as a off-white solid (1.12 gr, Yield: 91 %, Combined purity of both isomers by HPLC and mass spectrometry: >99%).

Synthesis of Adenosine, 5'-*S*-methyl-5'-thio-, 2',3'-diacetate (Compound #68):

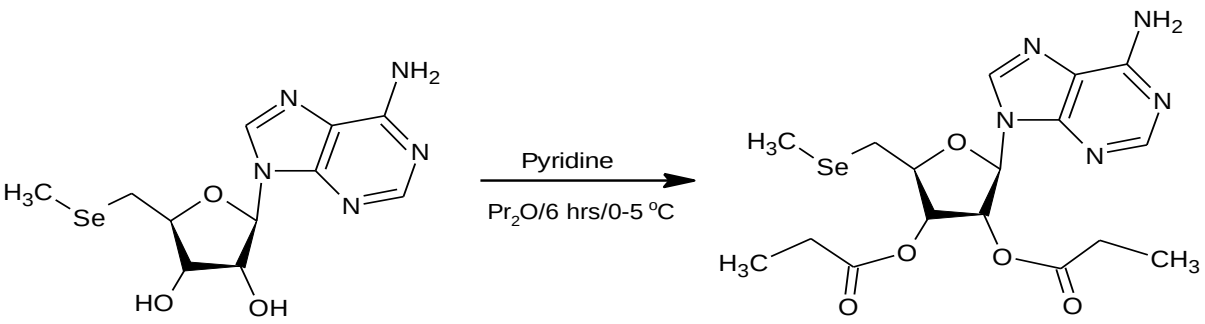
Scheme:



Synthetic procedure: 5'-*S*-methyl-5'-thio- Adenosine (1.0 gr, 0.0033 mole, 1.0 mole eq.) and anhydrous pyridine (10 ml) were placed in an oven dried, 50 ml three neck flask, equipped with a dropping funnel, inert gas inlet/outlet and a thermometer. The reaction set was placed in an ice/salt bath and agitation was initiated. When the temperature of the solution dropped to 0°C, acetic anhydride (10 ml, 0.105 mole, 31.8 mole eq.) was added drop-wise for 15 minutes and the temperature of the reaction mixture was maintained below 5°C during acetic anhydride addition. The reaction mixture was stirred for 6 hours at 5-10°C. The excess acetic anhydride was quenched by adding ice-cold water (100 ml), and then pH adjusted to 7 by adding 10 wt% $NaHCO_3$ aqueous solution. The aqueous mixture was extracted with ethyl acetate (2 x 100 ml). The combined ethyl acetate extracts were dried over anhydrous Na_2SO_4 (1gr) and filtered into a 250 ml round-bottomed flask. The filtrate was concentrated to dryness under reduced pressure at 35-40°C to give the crude product as a pale yellow syrupy liquid. Pure product was then obtained as off-white crystals (1.08 gr, Yield: 87 %, Purity by HPLC and mass spectrometry: >99%) by passing the yellow liquid through a silica gel column with a mixture of ethyl acetate and hexane (1:3 v/v).

Synthesis of Adenosine, 5'-*Se*-methyl-5'-seleno-, 2',3'-dipropionate (Compound #69):

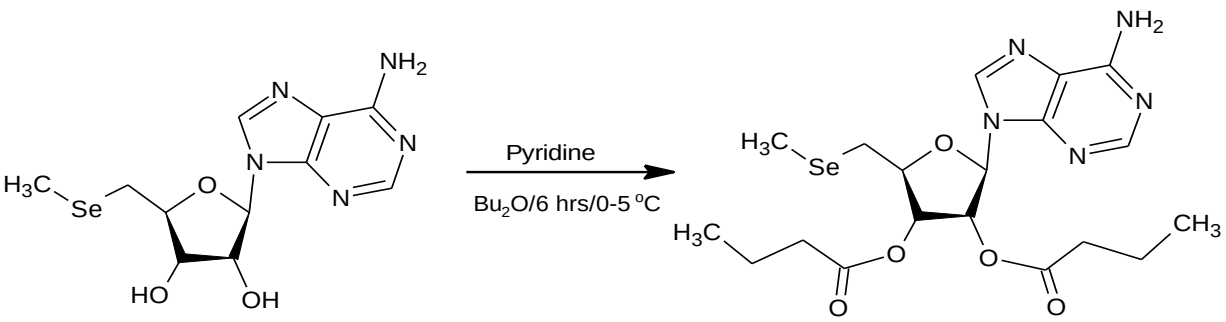
Scheme:



Synthetic procedure: 5'-*Se*-methyl-5'-seleno-Adenosine (1.0 gr, 0.0029 mole, 1.0 mole eq.) and anhydrous pyridine (10 ml) were placed in an oven dried, 50 ml three neck flask, equipped with a dropping funnel, inert gas inlet/outlet and a thermometer. The reaction set was placed in an ice/salt bath and agitation was initiated. When the temperature of the solution dropped to 0°C, propionic anhydride (10 ml, 0.078 mole, 27.0 mole eq.) was added drop-wise for 15 minutes and the temperature of the reaction mixture was maintained below 5°C during propionic anhydride addition. The reaction mixture was stirred for 6 hours at 0-5°C. The excess propionic anhydride was quenched by adding ice-cold water (100 ml), and then pH adjusted to 7 by adding 10 wt% $NaHCO_3$ aqueous solution. The aqueous mixture was extracted with ethyl acetate (2 x 100 ml). The combined ethyl acetate extracts were dried over anhydrous Na_2SO_4 (1 gr), filtered into a 250 ml round-bottomed flask. The filtrate was concentrated to dryness under reduced pressure at 35-40°C to give the crude product as a pale yellow syrupy liquid. The pure product was obtained as off-white crystals (1.18 gr, Yield: 89.3 %, Purity by HPLC and mass spectrometry: >99%) by passing the yellow liquid through a silica gel column with a mixture of ethyl acetate and hexanes (1:3 v/v).

Synthesis of Adenosine, 5'-*Se*-methyl-5'-seleno-, 2',3'-dibutanoate (Compound #70):

Scheme:



Synthetic procedure: 5'-*Se*-methyl-5'-seleno-Adenosine (1.0 gr, 0.0029 mole, 1.0 mole eq.) and anhydrous pyridine (10 ml) were placed in an oven dried, 50 ml three neck flask, equipped with a dropping funnel, inert gas inlet/outlet and a thermometer. The reaction set was placed in an ice/salt bath and agitation was initiated. When the temperature of the solution dropped to 0°C, butyric anhydride (10 ml, 0.078 mole, 27.0 mole eq.) was added drop-wise for 15 minutes and the temperature of the reaction mixture was maintained below 5°C during butyric anhydride addition. The reaction mixture was stirred for 6 hours at 0-5°C. The excess butyric anhydride was quenched by adding ice-cold water (100 ml), and then pH adjusted to 7 by adding 10 wt % $NaHCO_3$ aqueous solution. The aqueous mixture was extracted with ethyl acetate (2 x 100 ml). The combined ethyl acetate extracts were dried over anhydrous Na_2SO_4 (1 gr), filtered into a 250 ml round-bottomed flask. The filtrate was concentrated to dryness under reduced pressure at 35-40°C to give the crude product as a pale yellow syrupy liquid. The pure product was obtained as off-white crystals (1.20 gr, Yield: 85.7 %, Purity by HPLC and mass spectrometry: >99%) by passing the yellow liquid through a silica gel column with a mixture of ethyl acetate and hexanes (1:3 v/v).