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(72) Inventor; and

(71) Applicant : COOPER, Garth [NZ/NZ]; 2 Crummer Road, Ponsonby, Auckland, 1021 (NZ).

(74) Agent: AJ PARK; Level 22, State Insurance Tower, 1 Willis Street, Wellington (NZ).

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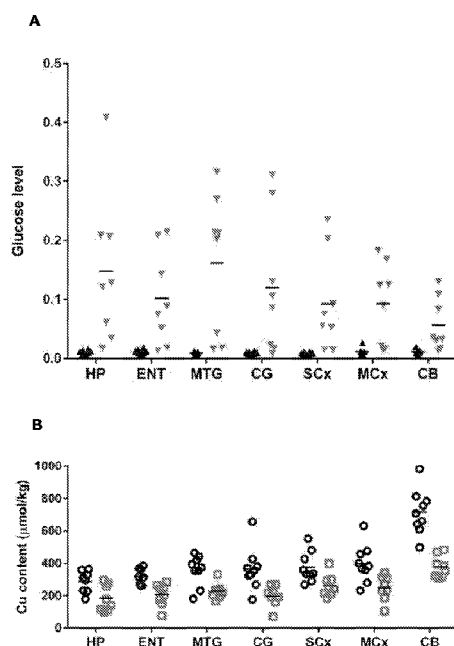
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FIGURE 1



(57) Abstract: Pharmaceutical compositions and methods for the treatment of subjects, including humans, who have, or are at risk for, Alzheimer's disease comprising tetramine copper chelators in combination with an agent effective to reduce one or more of encephalic glucose, encephalic sorbitol or encephalic fructose, such as amylin, GLP-1 agonists and DPP-IV inhibitors.

TREATMENT OF NEURODEGENERATIVE DISORDERS

FIELD OF THE INVENTION

5 **[0001]** The present invention relates generally to compounds, compositions and methods of treatment, and include compounds, compositions and methods for treating Alzheimer's disease, for improving physiological and/or neurological deficits associated with Alzheimer's disease.

BACKGROUND TO THE INVENTION

10 **[0002]** The following includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art, or relevant, to the presently described or claimed invention, or that any publication or document that is specifically or implicitly referenced is prior art.

15 **[0003]** Alzheimer's disease (AD) is an age-related neurodegenerative disease associated with pathological characteristics of senile plaques and neurofibrillary tangles. It is the most common form of dementia in humans, with a progression from episodic memory problems to a general decline in cognitive function. In 2013, approximately 44 million people were estimated to be affected by dementia.

[0004] A number of mechanisms have been proposed as causative of AD. As yet, however, no curative treatment with proven disease modifying effects exists.

20 **[0005]** Current treatments for AD are directed to treating cognitive and behavioural symptoms. Two types of drugs are currently used to treat cognitive symptoms of AD; cholinesterase inhibitors, which provide the neurotransmitter acetylcholine that is commonly depleted in the AD brain and thereby purportedly improve cell-to-cell communication; and memantine hydrochloride (Namenda™),
25 which is believed to inhibit signal transduction of N-methyl-d-aspartate (NMDA) receptors, and reportedly slows the progression of symptoms in moderate to severe AD.

[0006] Other medications, such as antidepressants, are sometimes used to help control the behavioral symptoms associated with AD.

5 [0007] There are no known approved treatments that are directed to the cause of AD, the underlying dysfunction or the resulting cell and tissue damage. Clearly there is a need for compounds and methods that limit or prevent damage associated with or causative of AD, as well as damage to organelles, cells and tissues that occurs as a consequence of AD.

10 [0008] There is also a need for compounds and methods that limit or prevent damage to cells and tissues that occurs directly or indirectly as a result of necrosis and/or inappropriate apoptosis associated with or causative of AD. Agents and methods that maintain neuronal and glial cell integrity represent novel protective agents with utility in limiting AD and AD-related dysfunction.

15 [0009] Copper chelation in combination with treatment to lower encephalic glucose, encephalic sorbitol, and/or encephalic fructose is proposed herein to treat AD. Triethylenetetramine dihydrochloride, a chelating compound for removal of excess copper from the body, is prescribed for Wilson's disease patients who cannot tolerate penicillamine. Triethylenetetramine dihydrochloride is *N,N'*-bis(2-aminoethyl)-1,2-ethanediamine dihydrochloride. It is a white to pale yellow crystalline hygroscopic powder. Syprine® (triethylenetetramine dihydrochloride) is available as 250 mg capsules for oral administration. See Siegemund R, *et al.*, "Mode of action of triethylenetetramine dihydrochloride on copper metabolism in Wilson's disease," *Acta Neurol. Scand.* **83**(6):364-6 (June 1991).

20 [0010] U.S. Patent Nos. 6,610,693, 6,348,465 and 6,951,890 provide copper chelators and other agents (*e.g.*, zinc which prevents copper absorption) to decrease copper values for the benefit of subjects suffering from diabetes and its complications. See also, Cooper, G.J., *et al.*, "Treatment of diabetes with copper binding compounds," U.S. Pat. App. No. 2005/0159489, published July 21, 2005; Cooper, G.J., *et al.*, "Copper antagonist compounds," U.S. Pat. App. No. 2005/0159364, published July 21, 2005; Cooper, G.J., *et al.*, "Preventing and/or treating cardiovascular disease and/or associated heart failure," U.S. Pat. App. No. 2003/0203973, published October 30, 2003. These also relate to therapies using copper antagonists, including triethylenetetramine, for example. Various experimental and clinical results are described in Cooper, G.J., *et al.*, "Regeneration of the heart in diabetes mellitus by selective copper chelation," *Diabetes* 53:2501-2508 (2004). See also Cooper, G.J., *et al.*, "Demonstration of a Hyperglycemia-Driven Pathogenic Abnormality of Copper Homeostasis in Diabetes and Its Reversability by Selective Chelation: Quantitative Comparisons Between the Biology

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of Copper and Eight Other Nutritionally Essential Elements in Normal and Diabetic Subjects," *Diabetes* **54**:1468-1476 (2005).

5 **[0011]** It is an object of the invention to go at least some way to fulfilling these needs and provide other related advantages, and/or to at least provide the public with a useful choice. Those skilled in the art will recognize further advantages and benefits of the invention after reading the disclosure.

SUMMARY OF THE INVENTION

10 **[0012]** The invention described and claimed herein have many attributes and embodiments including, but not limited to, those set forth or described or referenced in this Brief Summary. It is not intended to be all-inclusive and the invention described and claimed herein are not limited to or by the features or embodiments identified in this Brief Summary, which is included for purposes of illustration only and not restriction.

15 **[0013]** The present invention relates generally to compounds, compositions and methods for treating Alzheimer's disease (AD).

20 **[0014]** The present invention is directed in part to the treatment of AD by administration to a mammalian subject in need thereof an effective amount of a copper binding tetramine compound and an effective amount of an agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject.

[0015] The present invention is also directed in part to the treatment of AD by administration to a mammalian subject in need thereof an effective amount of

25 a) a compound according to Formula (I) or Formula (II), and
 b) an agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject.

30 **[0016]** In various embodiments, the copper binding tetramine compound binds Cu(II), for example, is a tetramine compound that is specific for Cu(II) over Cu(I). Particularly contemplated tetramine compounds include triethylenetetramine (2,2,2 tetramine), 2,3,2 tetramine and 3,3,3 tetramine as well as salts, active metabolites, derivatives, and prodrugs thereof.

[0017] In one embodiment, the tetramine compound is triethylenetetramine disuccinate.

5 **[0018]** In various embodiments, the agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject is selected from the group comprising amylin, an amylin analogue, a GLP-1 agonist, and a selective dipeptidyl peptidase (DPP-IV) inhibitor.

[0019] In one example, the amylin analogue is Symlin.

10 **[0020]** In various examples, the GLP-1 agonist is exenatide, liraglutide, lixisenatide, albiglutide, or dulaglutide, or any combination of two or more thereof.

[0021] In various examples, the selective dipeptidyl peptidase (DPP-IV) inhibitor is selected from the group comprising Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Anagliptin, Teneligliptin, Alogliptin, Trelagliptin, Gemigliptin, Dutogliptin, and Omarigliptin.

15 **[0022]** In one example, the selective dipeptidyl peptidase (DPP-IV) inhibitor is selected from the group comprising alogliptin, linagliptin, saxagliptin, sitagliptin, Nesina, Tradjenta, Onglyza, and Januvia.

20 **[0023]** Administration of the copper binding tetramine compound and of the agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject may be simultaneous, sequential, or separate.

[0024] In one embodiment, administration is of triethylenetetramine disuccinate and amylin or Symlin, and may be simultaneous, sequential, or separate administration.

25 **[0025]** In one embodiment, administration is of triethylenetetramine disuccinate and one or more agents selected from the group comprising Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Anagliptin, Teneligliptin, Alogliptin, Trelagliptin, Gemigliptin, Dutogliptin, and Omarigliptin, and may be simultaneous, sequential, or separate administration.

30 **[0026]** In one embodiment, administration is of triethylenetetramine disuccinate and one or more agents selected from the group comprising alogliptin, linagliptin,

saxagliptin, sitagliptin, Nesina, Tradjenta, Onglyza, and Januvia, and may be simultaneous, sequential, or separate administration.

5 **[0027]** Similarly, administration of the one or more compounds of Formula (I), and/or the one or more compounds of Formula (II), and of the agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject, may be simultaneous, sequential, or separate

10 **[0028]** In still further embodiments, methods are provided for treating AD by administering

10 a) one or more copper binding tetramine compounds, one or more compounds of Formula (I), and/or one or more compounds of Formula (II), and

10 b) one or more agents effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject,

15 in the form of one or more pharmaceutical compositions.

15 **[0029]** Thus, pharmaceutical compositions are also provided comprising one or more copper binding tetramine compounds, compounds of Formula (I), or compounds of Formula (II), additionally comprising or formulated to be administered in conjunction in with an agent effective to reduce the amount or concentration of

20 one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject, wherein the one or more pharmaceutical compositions comprises a pharmaceutically acceptable carrier or diluent.

25 **[0030]** In the context of the invention, AD includes tissue damage or degeneration associated with, caused by, or causative of AD, including damage or degeneration in which free radical mediated oxidative injury is involved, and damage or degeneration associated with AD in which cells inappropriately undergo apoptosis. Thus, treatment or amelioration of symptoms of AD, including cognitive and behavioural symptoms, are particularly contemplated.

30 **[0031]** In various embodiments, the mammalian subject is selected from the group consisting of a human, a domestic and farm animal, and zoo, sports, or pet animal, such as a dog, a horse, a cat, a sheep, a pig, a cow, or a deer. In a particularly contemplated example, the mammalian subject is a human.

30 **[0032]** In various embodiments, the mammalian subject is a non-diabetic subject, for example, a non-diabetic human subject, or a human subject who is not

undergoing treatment for diabetes. In various embodiments, the mammalian subject does not suffer from Wilson's disease.

5 **[0033]** In one embodiment, the reduction in the amount or concentration of encephalic glucose, encephalic sorbitol, or encephalic fructose is by a statistically significant amount. In one example, the reduction in the amount or concentration of encephalic glucose, encephalic sorbitol, or encephalic fructose is to an amount or concentration substantially equivalent to the amount or concentration of encephalic glucose, encephalic sorbitol, or encephalic fructose, respectively, found in a mammalian subject of the same species not suffering from AD.

10 **[0034]** In one embodiment, the reduction in the amount or concentration of encephalic glucose is to an amount or concentration substantially equivalent to the amount or concentration of encephalic glucose found in a mammalian subject of the same species not suffering from AD.

15 **[0035]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces glucose in the hippocampus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the hippocampus of a mammalian subject of the same species not suffering from AD.

20 **[0036]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces glucose in the entorhinal cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the entorhinal cortex of a
25 mammalian subject of the same species not suffering from AD.

30 **[0037]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces glucose in the middle-temporal gyrus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the middle-temporal gyrus of a mammalian subject of the same species not suffering from AD.

[0038] In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces glucose in the sensory

cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the sensory cortex of a mammalian subject of the same species not suffering from AD.

5 **[0039]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces glucose in the motor cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the motor cortex of a mammalian subject of the same species not suffering from AD.

10 **[0040]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces glucose in the cingulate gyrus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the cingulate gyrus of a mammalian subject of the same species not suffering from AD.

15 **[0041]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces glucose in the cerebellum. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the cerebellum of a mammalian subject of the same species not suffering from AD.

20 **[0042]** In one embodiment, the reduction in the amount or concentration of encephalic sorbitol is to an amount or concentration substantially equivalent to the amount or concentration of encephalic sorbitol found in a mammalian subject of the same species not suffering from AD.

25 **[0043]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the hippocampus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the hippocampus of a mammalian subject of the same species not suffering from AD.

30 **[0044]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the

entorhinal cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the entorhinal cortex of a mammalian subject of the same species not suffering from AD.

5 **[0045]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the middle-temporal gyrus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the middle-temporal gyrus of a mammalian subject of the same species not suffering from AD.

10 **[0046]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the sensory cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the sensory cortex of a mammalian subject of the same species not suffering from AD.

15 **[0047]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the motor cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the motor cortex of a mammalian subject of the same species not suffering from AD.

20 **[0048]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the cingulate gyrus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the cingulate gyrus of a mammalian subject of the same species not suffering from AD.

25 **[0049]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the cerebellum. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the cerebellum of a mammalian subject of the same species not suffering from AD.

30 **[0049]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces sorbitol in the cerebellum. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the cerebellum of a mammalian subject of the same species not suffering from AD.

[0050] In one embodiment, the reduction in the amount or concentration of encephalic fructose is to an amount or concentration substantially equivalent to the amount or concentration of encephalic fructose found in a mammalian subject of the same species not suffering from AD.

5 **[0051]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces fructose in the hippocampus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the hippocampus of a mammalian subject of
10 the same species not suffering from AD.

[0052] In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces fructose in the entorhinal cortex. In one embodiment, the reduction is to an amount or
15 concentration substantially equivalent to that found in the entorhinal cortex of a mammalian subject of the same species not suffering from AD.

[0053] In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces fructose in the middle-
20 temporal gyrus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the middle-temporal gyrus of a mammalian subject of the same species not suffering from AD.

[0054] In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces fructose in the sensory
25 cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the sensory cortex of a mammalian subject of the same species not suffering from AD.

[0055] In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces fructose in the motor
30 cortex. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the motor cortex of a mammalian subject of the same species not suffering from AD.

5 **[0056]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces fructose in the cingulate gyrus. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the cingulate gyrus of a mammalian subject of the same species not suffering from AD.

10 **[0057]** In one embodiment, administration of the one or more agents effective to reduce the amount or concentration of encephalic glucose, encephalic sorbitol, and/or encephalic fructose in the mammalian subject reduces fructose in the cerebellum. In one embodiment, the reduction is to an amount or concentration substantially equivalent to that found in the cerebellum of a mammalian subject of the same species not suffering from AD.

[0058] Diagnostic or prognostic methods for determining the presence or likelihood of developing AD are also provided.

15 **[0059]** In one embodiment, the invention relates to a method of assessing a mammalian subject's risk of developing AD which comprises:

 analysing a encephalic sample from said subject to determine the amount or concentration of glucose, fructose, or sorbitol in the sample, and
 comparing the amount or concentration of glucose, fructose, or sorbitol in the sample
20 to the amount or concentration of glucose, fructose, or sorbitol found in a mammalian subject of the same species not suffering from AD,
 wherein an elevated amount or concentration of encephalic glucose, encephalic sorbitol, or encephalic fructose in the sample is indicative of an increased risk of developing AD.

25 **[0060]** In one embodiment, an elevated concentration of encephalic glucose in a sample from a human subject indicative of an increased risk of developing AD is above about 10 μmol glucose/g wet weight.

[0061] In one embodiment, the invention relates to a method of diagnosing AD in a mammalian subject, the method comprising
30 analysing a encephalic sample from said subject to determine the amount or concentration of glucose, fructose, or sorbitol in the sample, and
 comparing the amount or concentration of glucose, fructose, or sorbitol in the sample to the amount or concentration of glucose, fructose, or sorbitol found in a mammalian subject of the same species not suffering from AD,

wherein an elevated amount or concentration of encephalic glucose, encephalic sorbitol, or encephalic fructose in the sample is indicative of the presence of AD in the mammalian subject.

5 **[0062]** In one embodiment, an elevated concentration of encephalic glucose in a sample from a human subject indicative of the presence of AD is above about 10 μ mol glucose/g wet weight.

10 **[0063]** In a further aspect, the invention relates to the use of
a) one or more copper binding tetramine compounds, one or more compounds of Formula (I), and/or one or more compounds of Formula (II), and
b) one or more agents effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject,
in the treatment of AD.

15 **[0064]** In another aspect, the invention relates to the use of
a) one or more copper binding tetramine compounds, one or more compounds of Formula (I), and/or one or more compounds of Formula (II), and
b) one or more agents effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject,
20 in the preparation of a medicament for the treatment of AD.

25 **[0065]** In various embodiments, the invention concerns pharmaceutical compositions containing such agents, articles and kits and delivery devices containing such agents, and tablets and capsules and formulations comprising such agents or compositions. Particularly contemplated are articles, kits and delivery devices enabling the separate, sequential or simultaneous administration of (a) the one or more copper binding tetramine compounds, one or more compounds of Formula (I), and/or one or more compounds of Formula (II), and of (b) the one or more agents effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in a mammalian
30 subject.

[0066] Pharmaceutical compositions also comprise a pharmaceutically acceptable carrier or diluent.

35 **[0067]** Useful copper chelating compounds include pharmaceutically acceptable polyamines, including copper-binding polyamines. Polyamines may include, for example, spermidine, as well as spermine and other tetramines. Tetramines also

include, for example, triethylenetetramine (for example, 2,2,2 tetramine or 2,3,2 tetramine), as well as salts, active metabolites, derivatives, and prodrugs thereof. Salts include, for example, triethylenetetramine hydrochloride salts (*e.g.*, triethylenetetramine dihydrochloride, triethylenetetramine tetrahydrochloride,) and succinate salts (*e.g.*, triethylenetetramine disuccinate), as well as maleate salts (*e.g.*, triethylenetetramine tetramaleate) and fumarate salts (*e.g.*, triethylenetetramine tetrafumarate). Metabolites include, for example, acetylated metabolites, such as N-acetyl triethylenetetramine (*e.g.*, monoacetyl-triethylenetetramine). Derivatives include, for example, PEG-modified tetramines, including PEG-modified triethylenetetramines. Other useful compounds include pharmaceutically acceptable compounds of Formula I and Formula II herein. Suitable copper antagonists include, for example, penicillamine, N-methylglycine, N-acetylpenicillamine, tetrathiomolybdate, 1,8-diamino-3, 6, 10, 13, 16, 19-hexa-azabicyclo[6.6.6]icosane, N,N'-diethyldithiocarbamate, bathocuproinedisulfonic acid, and bathocuprinedisulfonate.

[0068] Other suitable copper chelating compounds include, for example, pharmaceutically acceptable linear or branched tetramines capable of binding copper.

[0069] Such compounds may be administered in amounts, for example, that are effective to chelate encephalic Cu(II). Such compositions include, for example, tablets, capsules, solutions and suspensions for parenteral and oral delivery forms and formulations.

[0070] In various embodiments, the therapeutic methods described herein are carried out in conjunction with dietary or lifestyle modifications. The simplest of these regimens can be the provision to a subject with AD of motivation to implement such a lifestyle change, for example, dietary adjustments to reduce foods contributing to elevated brain urea concentration observed in AD, such as high protein foods.

[0071] These and other aspects of the invention, which are not limited to or by the information in this Brief Summary, are provided below.

[0072] It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7).

5 [0073] This invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

10 [0074] In this specification, where reference has been made to external sources of information, including patent specifications and other documents, this is generally for the purpose of providing a context for discussing the features of the present invention. Unless stated otherwise, reference to such sources of information is not to be construed, in any jurisdiction, as an admission that such sources of information are prior art or form part of the common general knowledge in the art.

15 [0075] The term "comprising" as used in this specification means "consisting at least in part of". When interpreting statements in this specification which include that term, the features, prefaced by that term in each statement, all need to be present but other features can also be present. Related terms such as "comprise" and "comprised" are to be interpreted in the same manner.

BRIEF DESCRIPTION OF THE DRAWINGS

20 [0076] The invention will now be described by way of example only and with reference to the drawings in which:

25 [0077] Figure 1 shows regional A) brain copper, and B) mean glucose levels in the cerebellum (CB), cingulate gyrus (CG), entorhinal cortex (ENT), hippocampus (HP), motor cortex (MCx), middle temporal gyrus (MTG), and sensory cortex (SCx) of patients with AD (n=7-9/region; on right) and control patients (n=7-9/region on left).

DETAILED DESCRIPTION OF THE INVENTION

[0078] The present invention combines copper chelation with treatment to lower levels of glucose, fructose and/or sorbitol in the brain to treat or prevent AD.

30 [0079] As presented in the Examples, the applicant has demonstrated that 1) levels of glucose, sorbitol and fructose are elevated across many regions of the AD brain, and 2) the AD brain is copper-deficient.

[0080] Without wishing to be bound by any theory, the applicant believes that elevated levels of glucose, fructose and sorbitol in the brain are likely to be toxic via the formation of advanced glycation endproducts (AGEs) such as N-epsilon-carboxy-methyllysine (CML).

5 **[0081]** The formation of AGEs in the brain creates pathogenic copper (II) binding sites in the extracellular matrix (ECM) (Kamalov et al., 2015. *Org. Biomol. Chem.* 14:13(1): 3058-3063). Copper bound to AGEs such as CML is catalytically active and therefore toxic.

10 **[0082]** The applicant believes that ECM-bound copper (II) causes deficient neuronal copper uptake via suppression of membrane-bound copper uptake mediated by copper transporter CTR1 and intracellular copper transport via copper chaperones (see Zhang et al., 2014 *Cardiovascular Diabetology* 13:100).

15 **[0083]** It has been shown that CML-bound copper itself catalyses glucose-mediated AGE formation in a feed-forward reaction (Brings et al., 2015. *Biochim. Biophys. Acta* 1852(8): 1610-1618).

20 **[0084]** Physiological copper cations are crucial to the function of enzymes involved in processes including tissue antioxidant defence, suppression of inflammation and effective utilisation of metabolic fuels. Enzymes that are targeted and repaired by copper chelation include superoxide dismutase I (SOD1), superoxide dismutase III (SOD3), cytochrome c oxidase subunit I (COI) and cytochrome c oxidase subunit II (COII).

25 **[0085]** Reduction of copper available to be incorporated into these enzymes as seen in the AD brain results in impaired function of the enzymes, which may result in mitochondrial dysfunction. For example, a reduction in cytochrome c oxidase activity leads to increased electron leaking from the mitochondrial and oxidative stress.

30 **[0086]** The combination of copper chelation and glucose-lowering treatment inhibits AGE formation and binding of copper to AGEs such as CML, thus reducing AGE-bound copper-induced suppression of copper transport in the brain. The method of the invention thus suppresses AGE formation, ameliorates copper deficiency and restores enzyme and mitochondrial function in the brain.

[0087] As used herein, a "copper antagonist" is a pharmaceutically acceptable compound that binds or chelates copper *in vivo* for removal. Copper chelators are presently preferred copper antagonists. Copper (II) chelators, and copper (II)

specific chelators (*i.e.*, those that preferentially bind copper (II) over other forms of copper such as copper (I)), are especially preferred.

[0088] As used herein, "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids the like. When a compound is basic, for example, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include, for example, acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid, and the like. Particularly preferred are hydrochloric and succinic acid copper antagonist salts. Succinic acid copper antagonist salts are most preferred, particularly for those copper antagonist salts that are not anhydrous.

[0089] As used herein, "preventing" means preventing in whole or in part, or ameliorating or controlling.

[0090] As used herein, a "therapeutically effective amount" in reference to the compounds or compositions of the instant invention refers to the amount sufficient to induce a desired biological, pharmaceutical, or therapeutic result. That result can be alleviation of the signs, symptoms, or causes of a disease or disorder or condition, or any other desired alteration of a biological system. In one aspect of the present inventions, the result will involve the prevention, decrease, or reversal of AD, in whole or in part, and prevention and/or treatment of related conditions, including those referenced herein.

[0091] As used herein, the term "treating" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to having the disorder, or those diagnosed with the disorder, or those in which the disorder is to be prevented.

[0092] As used herein, encephalic glucose refers the glucose present in the brain and/or encephalic cavity of a mammalian subject, and particularly contemplates glucose present in one or more tissues or regions of the brain. Methods to determine the amount or concentration of encephalic glucose are known in the art, and exemplary methods are provided herein in the Examples.

5 **[0093]** As used herein, encephalic fructose refers the glucose present in the brain and/or encephalic cavity of a mammalian subject, and particularly contemplates fructose present in one or more tissues or regions of the brain. Methods to determine the amount or concentration of encephalic fructose are known in the art, and exemplary methods are provided herein in the Examples.

10 **[0094]** As used herein, encephalic sorbitol refers the glucose present in the brain and/or encephalic cavity of a mammalian subject, and particularly contemplates sorbitol present in one or more tissues or regions of the brain. Method to determine the amount or concentration of encephalic sorbitol are known in the art, and exemplary methods are provided herein in the Examples.

[0095] It will be appreciated that agents that are effective to lower encephalic glucose, sorbitol, or fructose without a concomitant risk of hypoglycaemia are preferred. Representative examples of such agents are discussed below.

Amylin and amylin analogues

15 **[0096]** Amylin is a small peptide hormone released into the bloodstream by the pancreatic β -cells after a meal, and reportedly modulates blood glucose by slowing gastric emptying, promoting satiety, and inhibiting inappropriate secretion of glucagon. Amylin analogues, such as symlin (Pramlintide™, AstraZeneca), are used to augment endogenous amylin, or to replace amylin's function in diabetics who do
20 not naturally produce amylin.

GLP-1 agonists

[0097] GLP-1 is a naturally-occurring peptide that is released within minutes of eating a meal. It has been reported to suppress glucagon secretion from pancreatic alpha cells and stimulate insulin secretion by pancreatic beta cells. GLP-1 receptor
25 agonists are generally used in the treatment of type 2 diabetes. Representative GLP-1 agonists suitable for use in the methods and compositions described herein include exenatide (Byetta™/Bydureon™, AstraZeneca, approved in 2005/2012), liraglutide (Victoza™, Saxenda™, Novo Nordisk, FDA approved 2010), lixisenatide (Lyxumia™, Zealand Pharma & Sanofi, approved in EU 2013), albiglutide (Eperzan™, Tanzeum™,
30 GSK, FDA approved in 2014), and dulaglutide (Trulicity™, Eli Lilly, FDA approved in 2014).

Selective dipeptidyl peptidase (DPP-IV) inhibitors

[0098] Dipeptidyl peptidase (DPP-IV) inhibitors have been reported to reduce glucagon and blood glucose levels via increasing insulin levels. Representative DPP-IV inhibitors suitable for use in the method and compositions described herein include Sitagliptin (FDA approved 2006, marketed by Merck & Co. as Januvia™), Vildagliptin (EU approved 2007, marketed in the EU by Novartis as Galvus™), Saxagliptin (FDA approved in 2009, marketed as Onglyza™), Linagliptin (FDA approved in 2011, marketed as Tradjenta™ by Eli Lilly Co and Boehringer Ingelheim), Anagliptin (approved in Japan in 2012, marketed by Sanwa Kagaku Kenkyusho Co., Ltd. and Kowa Company, Ltd.), Teneligliptin (approved in Japan in 2012), Alogliptin (FDA approved 2013, marketed by Takeda Pharmaceutical Company), Trelagliptin (approved for use in Japan in 2015), Gemigliptin (LG Life Sciences), Dutogliptin (Phenomix Corporation), and Omarigliptin (approved in Japan in 2015, developed by Merck & Co.).

15 Copper binding compounds

[0099] Suitable copper-chelating compounds include copper binding polyamine compound, polyamine compounds that bind Cu^{+2} , and preferably polyamine compounds that are specific for Cu^{+2} over Cu^{+1} . Polyamine compounds may include, for example, spermine, as well as spermidine and other tetramines. Preferred tetramine compounds include triethylenetetramine (2,2,2 tetramine), 2,3,2 tetramine and 3,3,3 tetramine as well as salts, active metabolites, derivatives, and prodrugs thereof. Other pharmaceutically acceptable polyamines are also contemplated.

[00100] Nitrogen-containing copper antagonists, for example, such as, for example, triethylenetetramine, that can be delivered as a salt(s) (such as acid addition salts, e.g., triethylenetetramine disuccinate or triethylenetetramine dihydrochloride) act as copper-chelating agents or antagonists, which aids the elimination of copper from the body by forming a stable soluble complex that is readily excreted by the kidney. Thus inorganic acids can be used, e.g., sulfuric acid, nitric acid, hydrohalic acids such as hydrochloric acid or hydrobromic acid, phosphoric acids such as orthophosphoric acid, sulfamic acid. This is not an exhaustive list. Other organic acids can be used to prepare suitable salt forms, in particular aliphatic, alicyclic, araliphatic, aromatic or heterocyclic mono-or polybasic carboxylic, sulfonic or sulfuric acids, (e.g., formic acid, acetic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, lactic acid, tartaric acid, malic acid, citric acid, gluconic acid, ascorbic acid, nicotinic

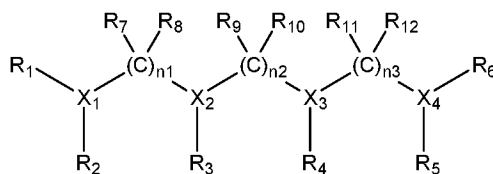
acid, isonicotinic acid, methanesulfonic acid, ethanesulfonic acid, ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, naphthalenemono-and-disulfonic acids, and laurylsulfuric acid). Hydrochloric acid and succinic acid salts are preferred, and succinic acid salts are most preferred.

Those in the art will be able to prepare other suitable salt forms.

[00101] Nitrogen-containing copper antagonists, for example, such as, for example, triethylenetetramine, can also be in the form of quarternary ammonium salts in which the nitrogen atom carries a suitable organic group such as an alkyl, alkenyl, alkynyl or aralkyl moiety. In one embodiment such nitrogen-containing copper antagonists are in the form of a compound or buffered in solution and/or suspension to a near neutral pH much lower than the pH 14 of a solution of triethylenetetramine itself.

[00102] Other copper antagonists include derivatives, for example, triethylenetetramine in combination with picolinic acid (2-pyridinecarboxylic acid). These derivatives include, for example, triethylenetetramine picolinate and salts of triethylenetetramine picolinate, for example, triethylenetetramine picolinate HCl. They also include, for example, triethylenetetramine di-picolinate and salts of triethylenetetramine di-picolinate, for example, triethylenetetramine di-picolinate HCl. Picolinic acid moieties may be attached to triethylenetetramine, for example one or more of the CH₂ moieties, using chemical techniques known in the art. Those in the art will be able to prepare other suitable derivatives, for example, triethylenetetramine-PEG derivatives, which may be useful for particular dosage forms including oral dosage forms having increased bioavailability.

[00103] Other compounds include cyclic and acyclic compounds according to the following formulae, for example:



FORMULA I

[00104] Tetra-heteroatom acyclic compounds within Formula I are provided where X₁, X₂, X₃, and X₄ are independently chosen from the atoms N, S or O, such that,

(a) for a four-nitrogen series, *i.e.*, when X_1 , X_2 , X_3 , and X_4 are N then: R_1 , R_2 , R_3 , R_4 , R_5 , and R_6 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 , n_2 , and n_3 are independently chosen to be 2 or 3; and, R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R_1 , R_2 , R_3 , R_4 , R_5 , or R_6 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein. Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , or R_{12} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(b) for a first three-nitrogen series, *i.e.*, when X_1 , X_2 , X_3 , are N and X_4 is S or O then: R_6 does not exist; R_1 , R_2 , R_3 , R_4 and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 , n_2 , and n_3 are independently chosen to be 2 or 3; and, R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R_1 , R_2 , R_3 , R_4 , or R_5 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10

alkyl-S-peptide, C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(c) for a second three-nitrogen series, *i.e.*, when X₁, X₂, and X₄ are N and X₃ is O or S then: R₄ does not exist and R₁, R₂, R₃, R₅, and R₆ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, and n₃ are independently chosen to be 2 or 3; and, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₁, R₂, R₃, R₅, or R₆ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(d) for a first two-nitrogen series, *i.e.*, when X₂ and X₃ are N and X₁ and X₄ are O or S then: R₁ and R₆ do not exist; R₂, R₃, R₄, and R₅ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, and n₃ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl,

fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₂, R₃, R₄, or R₅ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(e) for a second two-nitrogen series, *i.e.*, when X₁ and X₃ are N and X₂ and X₄ are O or S then: R₃ and R₆ do not exist; R₁, R₂, R₄, and R₅ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, and n₃ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₁, R₂, R₄, or R₅ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(f) for a third two-nitrogen series, *i.e.*, when X₁, and X₂ are N and X₃ and X₄ are O or S then: R₄ and R₆ do not exist; R₁, R₂, R₃, and R₅ are independently chosen from H, CH₃, C2-

C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, and n₃ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₁, R₂, R₃, or R₅ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(g) for a fourth two-nitrogen series, *i.e.*, when X₁ and X₄ are N and X₂ and X₃ are O or S then: R₃ and R₄ do not exist; R₁, R₂, R₅ and R₆ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, and n₃ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₁, R₂, R₅, or R₆ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins,

polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00105] Second, for a tetra-heteroatom series of cyclic analogues, one of R₁ and R₂ and one of R₅ and R₆ are joined together to form the bridging group (CR₁₃R₁₄)_{n4}, and X₁, X₂, X₃, and X₄ are independently chosen from the atoms N, S or O such that,

(a) for a four-nitrogen series, *i.e.*, when X₁, X₂, X₃, and X₄ are N then: R₂, R₃, R₄, and R₅ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, n₃, and n₄ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₂, R₃, R₄, or R₅ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ or R₁₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(b) for a three-nitrogen series, *i.e.*, when X₁, X₂, X₃, are N and X₄ is S or O then: R₅ does not exist; R₂, R₃, and R₄ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, n₃, and n₄ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ are independently chosen from

H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₂, R₃ or R₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half-lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ or R₁₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

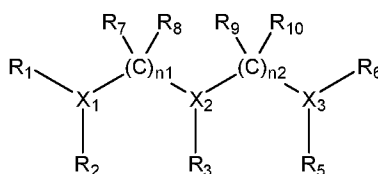
(c) for a first two-nitrogen series, *i.e.*, when X₂ and X₃ are N and X₁ and X₄ are O or S then: R₂ and R₅ do not exist; R₃ and R₄ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, n₃, and n₄ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or both of R₃, or R₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half-lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ or R₁₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-

C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(d) for a second two-nitrogen series, *i.e.*, when X_1 and X_3 are N and X_2 and X_4 are O or S then: R_3 and R_5 do not exist; R_2 and R_4 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 , n_2 , n_3 , and n_4 are independently chosen to be 2 or 3; and R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , R_{13} and R_{14} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or both of R_2 , or R_4 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half-lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , R_{13} or R_{14} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(e) for a one-nitrogen series, *i.e.*, when X_1 is N and X_2 , X_3 and X_4 are O or S then: R_3 , R_4 and R_5 do not exist; R_2 is independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 , n_2 , n_3 , and n_4 are independently chosen to be 2 or 3; and R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , R_{13} and R_{14} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, R_2 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the

constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ or R₁₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

**FORMULA II**

[00106] Tri-heteroatom compounds within Formula II are provided where X₁, X₂, and X₃ are independently chosen from the atoms N, S or O such that,

(a) for a three-nitrogen series, when X₁, X₂, and X₃ are N then: R₁, R₂, R₃, R₅, and R₆ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, and n₂ are independently chosen to be 2 or 3; and R₇, R₈, R₉, and R₁₀ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₁, R₂, R₃, R₅ or R₆ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, or R₁₀ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half-lives of the constructs. Examples of

such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(b) for a first two-nitrogen series, when X_1 and X_2 are N and X_3 is S or O then: R_3 does not exist; R_1 , R_2 , R_5 , and R_6 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 , and n_2 are independently chosen to be 2 or 3; and R_7 , R_8 , R_9 , and R_{10} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R_1 , R_2 , R_5 or R_6 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R_7 , R_8 , R_9 , or R_{10} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half-lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(c) for a second, two-nitrogen series, when X_1 and X_2 are N and X_3 is O or S then: R_5 does not exist; R_1 , R_2 , R_3 , and R_6 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 and n_2 are independently chosen to be 2 or 3; and R_7 , R_8 , R_9 , and R_{10} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R_1 , R_2 , R_5 , or R_6 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half

lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, or R₁₀ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half-lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00107] A series of tri-heteroatom cyclic analogues according to the above Formula II are provided in which R₁ and R₆ are joined together to form the bridging group (CR₁₁R₁₂)_{n3}, and X₁, X₂ and X₃ are independently chosen from the atoms N, S or O such that:

(a) for a three-nitrogen series, when X₁, X₂, and X₃ are N then: R₂, R₃, and R₅ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH); n₁, n₂, and n₃ are independently chosen to be 2 or 3; and R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or several of R₂, R₃, or R₅ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(b) for a two-nitrogen series, when X_1 and X_2 are N and X_3 is S or O then: R_5 does not exist; R_2 , and R_3 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 , n_2 , and n_3 are independently chosen to be 2 or 3; and R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, one or both of R_2 or R_3 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half-lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , or R_{12} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

(c) for a one-nitrogen series, when X_1 is N and X_2 and X_3 are O or S then:
 R_3 and R_5 do not exist; R_2 is independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$; n_1 , n_2 , and n_3 are independently chosen to be 2 or 3; and R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl. In addition, R_2 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10

alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein. Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00108] The compounds of the invention, including triethylenetetramine active agents, may be made using any of a variety of chemical synthesis, isolation, and purification methods known in the art.

[00109] In still further embodiments, methods are provided for treating AD by administering one or more copper binding tetramine compounds, compounds of Formula (I), or compounds of Formula (II), in the form of a pharmaceutical composition. Thus, pharmaceutical compositions are also provided comprising one or more copper binding tetramine compounds, compounds of Formula (I), or compounds of Formula (II), in combination with a pharmaceutically acceptable carrier or diluent.

[00110] Copper antagonists useful in the invention also include copper chelators that have been pre-complexed with a non-copper metal ion prior to administration for therapy. Metal ions used for pre-complexing have a lower association constant for the copper antagonist than that of copper. For example, a metal ion for pre-complexing a copper antagonist that chelates Cu²⁺ is one that has a lower binding affinity for the copper antagonist than Cu²⁺. Preferred metal ions for precomplexing include calcium (*e.g.*, Ca²⁺), magnesium (*e.g.*, Mg²⁺), chromium (*e.g.*, Cr²⁺ and Cr³⁺), manganese (*e.g.*, Mn²⁺), zinc (*e.g.*, Zn²⁺), selenium (*e.g.*, Se⁴⁺), and iron (*e.g.*, Fe²⁺ and Fe³⁺). Most preferred metal ions for precomplexing are calcium, zinc, and iron. Other metals include, for example, cobalt (*e.g.*, Co²⁺), nickel (*e.g.*, Ni²⁺), silver (*e.g.*, Ag¹⁺), and bismuth (*e.g.*, Bi³⁺). Metals are chosen with regard, for example, to their relative binding to the copper antagonist, and relative to toxicity and the dose of the copper antagonist to be administered.

[00111] Also encompassed are metal complexes comprising copper antagonists and non-copper metals (that have lower binding affinities than copper for the copper antagonist) and one or more additional ligands than typically found in complexes of

that metal. These additional ligands may serve to block sites of entry into the complex for water, oxygen, hydroxide, or other species that may undesirably complex with the metal ion and can cause degradation of the copper antagonist. For example, copper complexes of triethylenetetramine have been found to form pentacoordinate complexes with a tetracoordinated triethylenetetramine and a chloride ligand when crystallized from a salt solution rather than a tetracoordinate Cu^{2+} triethylenetetramine complex. In this regard, 219 mg of triethylenetetramine $\cdot$ 2 HCl were dissolved in 50 ml, and 170 mg of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ were dissolved in 25 ml ethanol (95%). After addition of the CuCl_2 solution to the triethylenetetramine solution, the color changed from light to dark blue and white crystals precipitated. The crystals were dissolved by addition of a solution of 80 mg NaOH in 15 ml H_2O . After the solvent was evaporated, the residue was dissolved in ethanol, and two equivalents of ammonium-hexafluorophosphate were added. Blue crystals could be obtained after reduction of the solvent. Crystals were found that were suitable for x-ray structure determination. X-ray crystallography revealed a $[\text{Cu}(\text{triethylenetetramine})\text{Cl}]$ complex. Other coordinated complexes may be formed from or between copper antagonists, for example, copper chelators (such as Cu^{2+} chelators, spermidine, spermine, tetracyclam, *etc.*), particularly those subject to degradative pathways such as those noted above, by providing additional complexing agents (such as anions in solution, for example, I^- , Br^- , F^- , $(\text{SO}_4)^{2-}$, $(\text{CO}_3)^{2-}$, BF_4^- , NO_3^- , ethylene, pyridine, *etc.*) in solutions of such complexes. This may be particularly desirable for complexes with more accessible metal ions, such as planar complexes or complexes having four or fewer coordinating agents, where one or more additional complexing agents could provide additional shielding to the metal from undesirable ligands that might otherwise access the metal and displace a desired complexing agent.

[00112] General synthetic chemistry protocols are somewhat different for these classes of molecules due to their propensity to chelate with metallic cations, including copper. Glassware should be cleaned and silanized prior to use. Plasticware should be chosen specifically to have minimal presence of metal ions. Metal implements such as spatulas should be excluded from any chemistry protocol involving chelators. Water used should be purified by sequential carbon filtering, ion exchange and reverse osmosis to the highest level of purity possible, not by distillation. All organic solvents used should be rigorously purified to exclude any possible traces of metal ion contamination.

[00113] Care must also be taken with purification of such derivatives due to their propensity to chelate with a variety of cations, including copper, which may be

present in trace amounts in water, on the surface of glass or plastic vessels. Once again, glassware should be cleaned and silanized prior to use. Plasticware should be chosen specifically to have minimal presence of metal ions. Metal implements such as spatulas should be avoided, and water used should be purified by sequential carbon filtering, ion exchange and reverse osmosis to the highest level of purity possible, and not by distillation. All organic solvents used should be rigorously purified to exclude any possible traces of metal ion contamination. Ion exchange chromatography followed by lyophilization is typically the best way to obtain pure solid materials of these classes of molecules. Ion exchange resins should be washed clean of any possible metal contamination.

[00114] Many of the synthetic routes allow for control of the particular R groups introduced. For synthetic methods incorporating amino acids, synthetic amino acids can be used to incorporate a variety of substituent R groups. The dichloroethane synthetic schemes also allow for the incorporation of a wide variety of R groups by using dichlorinated ethane derivatives. It will be appreciated that many of these synthetic schemes can lead to isomeric forms of the compounds; such isomers can be separated using techniques known in the art.

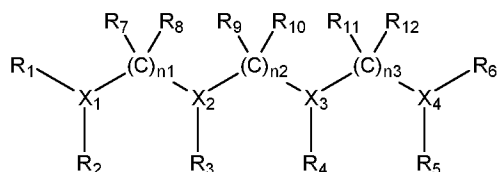
[00115] Documents describing aspects of these synthetic schemes include the following: (1) A W von Hoffman, *Berichte* 23, 3711 (1890); (2) The Polymerization Of Ethylenimine, Giffin D. Jones, Arne Langsjoen, Sister Mary Marguerite Christine Neumann, Jack Zomlefer, *J. Org. Chem.*, 1944; 9(2); 125-147; (3) The peptide way to macrocyclic bifunctional chelating agents: synthesis of 2-(p-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid and study of its yttrium(III) complex, Min K. Moi *et al.*, *J. Am. Chem. Soc.*, 1988; 110(18); 6266-6267; (4) Synthesis of a kinetically stable ⁹⁰Y labelled macrocycle-antibody conjugate, Jonathan P L Cox, *et al.*, *J. Chem. Soc. Chem. Comm.*, 797 (1989); (5) Specific and stable labeling of antibodies with technetium-99m with a diamide dithiolate chelating agent, Fritzberg AR, Abrams PG, Beaumier PL, Kasina S, Morgan AC, Rao TN, Reno JM, Sanderson JA, Srinivasan A, Wilbur DS, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* 85(11):4025-4029 (1988 Jun); (6) Towards tumour imaging with ¹¹¹In labelled macrocycle-antibody conjugates, Andrew S Craig *et al.*, *J. Chem. Soc. Chem. Comm.*, 794 (1989); (7) Synthesis of C- and N-functionalised derivatives of NOTA, DOTA, and DTPA: bifunctional complexing agents for the derivitisation of antibodies, Jonathan P L Cox *et al.*, *J. Chem. Soc. Perkin. I*, 2567 (1990); (8) Macrocyclic chelators as anticancer agents in radioimmunotherapy, N R A Beeley and P R J Ansell, *Current Opinions in Therapeutic Patents*, 2:1539-1553 (1992); and (9) Synthesis of new macrocyclic amino-phosphinic acid complexing agents and their C- and P-

functionalised derivatives for protein linkage, Christopher J Broan et al., Synthesis, 63 (1992).

[00116] Acyclic and cyclic compounds of the invention and exemplary synthetic methods and existing syntheses from the art include the following:

5

For tetra-heteroatom acyclic examples of Formula I:



X₁, X₂, X₃, and X₄ are independently chosen from the atoms N, S or O such that:

4N SERIES:

10

when X₁, X₂, X₃, and X₄ are N then:

R₁, R₂, R₃, R₄, R₅, and R₆ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH);

15

n₁, n₂, and n₃ are independently chosen to be 2 or 3, and each repeat of any of n₁, n₂, and n₃ may be the same as or different than any other repeat; and

R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

20

[00117] In addition, one or several of R₁, R₂, R₃, R₄, R₅, or R₆ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

25

30

[00118] Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene

glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

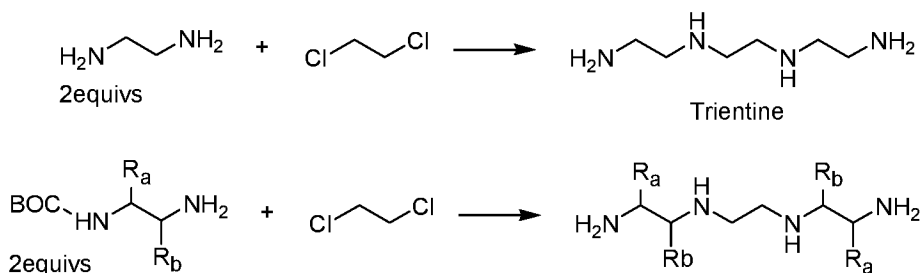
[00119] Also provided are embodiments wherein one, two, three or four of R1 through R12 are other than hydrogen.

10 **[00120]** In some embodiments, the compounds of Formula I or II are selective for a particular oxidation state of copper. For example, the compounds may be selected so that they preferentially bind oxidized copper, or copper (II). Copper selectivity can be assayed using methods known in the art. Competition assays can be done using isotopes of copper (I) and copper (II) to determine the ability of the compounds to selectively bind one form of copper.

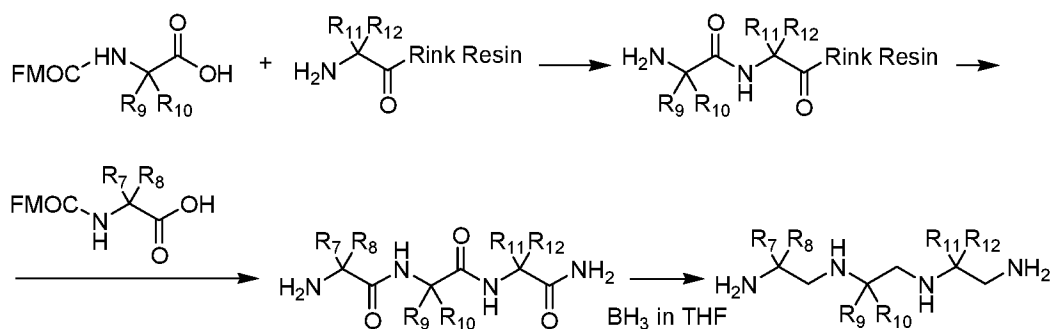
15 **[00121]** In some embodiments, the compounds of Formula I or II may be chosen to avoid excessive lipophilicity, for example by avoiding large or numerous alkyl substituents. Excessive lipophilicity can cause the compounds to bind to and/or pass through cellular membranes, thereby decreasing the amount of compound available for chelating copper, particularly for extracellular copper, which may be
20 predominantly in the oxidized form of copper (II).

Synthesis of examples of the open chain 4N series of Formula I

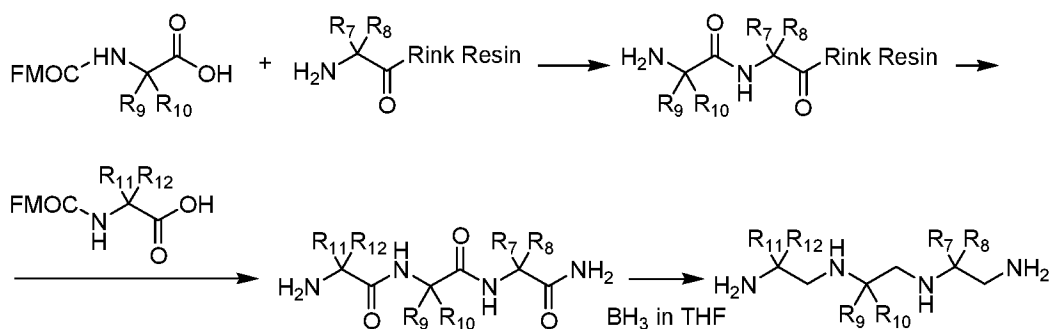
[00122] Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give triethylenetetramine directly (1). Modification of this procedure by using starting materials with appropriate Ra and Rb groups (where Ra, Rb = R7, R8 or R11, R12) would lead to symmetrically substituted open chain 4N examples as shown below:



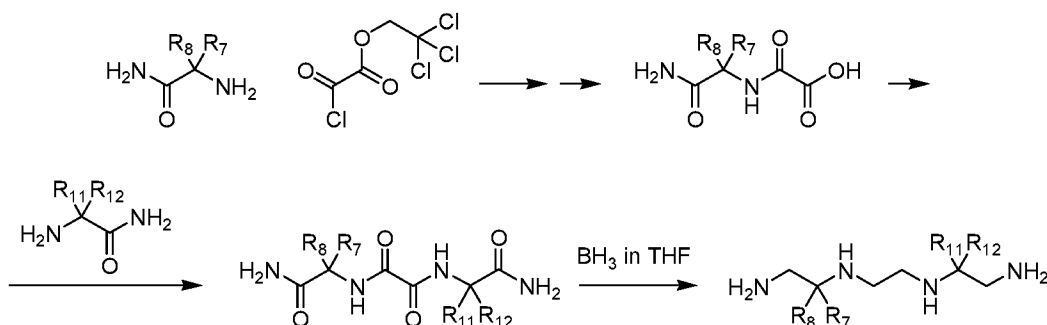
[00123] The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the tetra-aza series. In order to obtain the un-symmetrically substituted derivatives a variant of some chemistry described by Meares et al (3) should be used. Standard peptide synthesis using the Rink resin along with Fmoc protected natural and un-natural amino acids which can be conveniently cleaved at the penultimate step of the synthesis generates a tri-peptide C-terminal amide. This is reduced using Diborane in THF to give the open chain tetra-aza compounds as shown below:



[00124] The incorporation of R1, R2, R5 and R6 can be accomplished with this chemistry by standard procedures.



[00125] The reverse Rink approach, shown above, also leads to this class of tetra-aza derivatives and may be useful in cases where peptide coupling of a sterically hindered amino acid requires multiple coupling attempts in order to achieve success in the initial Rink approach.



[00126] The oxalamide approach, shown above, also can lead to successful syntheses of this class of compounds, although the central substituents are always going to be hydrogen or its isotopes with this kind of chemistry. This particular variant makes use of the trichloroethyl ester group to protect one of the carboxylic acid functions of oxalic acid but other protecting groups are also envisaged. Reaction of an amino acid amide derived from a natural or unnatural amino acid with a differentially protected oxalyl mono chloride gives the mono-oxalamide shown which can be reacted under standard peptide coupling condition to give the un-symmetrical bis-oxalamide which can then be reduced with diborane to give the desired tetra-aza derivative.

1. 3NX series 1:

when X_1, X_2, X_3 , are N and X_4 is S or O then:

R_6 does not exist

R_1, R_2, R_3, R_4 and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$;

n_1, n_2 , and n_3 are independently chosen to be 2 or 3, and each repeat of any of n_1, n_2 , and n_3 may be the same as or different than any other repeat; and

$R_7, R_8, R_9, R_{10}, R_{11}$, and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

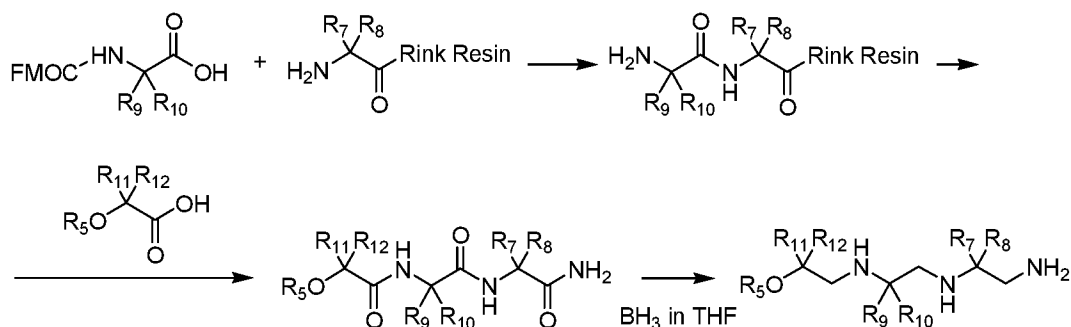
[00127] In addition, one or several of R_1, R_2, R_3, R_4 , or R_5 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-

kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

5 **[00128]** Furthermore one or several of R7, R8, R9, R10, R11, or R12 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10
10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

Synthesis of examples of the open chain 3NX series 1 of Formula I:

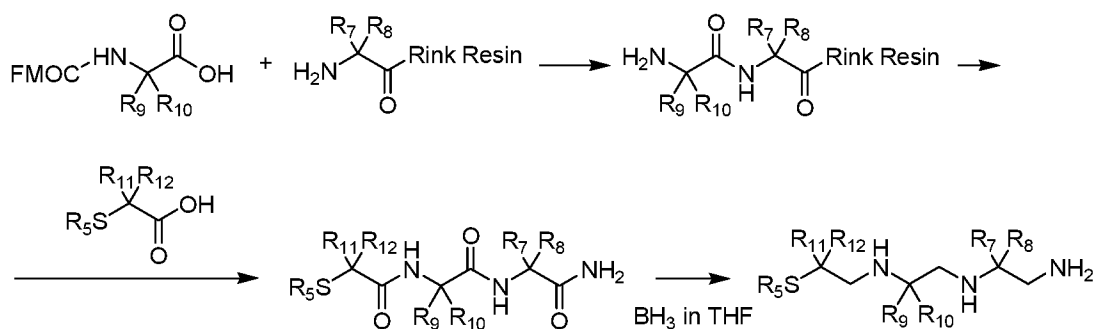
[00129] Variations of the syntheses used for the 4N series provide examples of the 3N series 1 class of compounds. The chemistry described by Meares et al (3) can
15 be modified to give examples of the 3NX series of compounds.



[00130] Standard peptide synthesis according to the so-called reverse Rink approach as shown above using Fmoc protected natural and un-natural amino acids which can be conveniently cleaved at the penultimate step of the synthesis generates
20 a modified tri-peptide C-terminal amide. The cases where X4 is O are incorporated by the use of an α-substituted carboxylic acid in the last coupling step. This is reduced using Diborane in THF to give the open chain tetra-aza compounds.

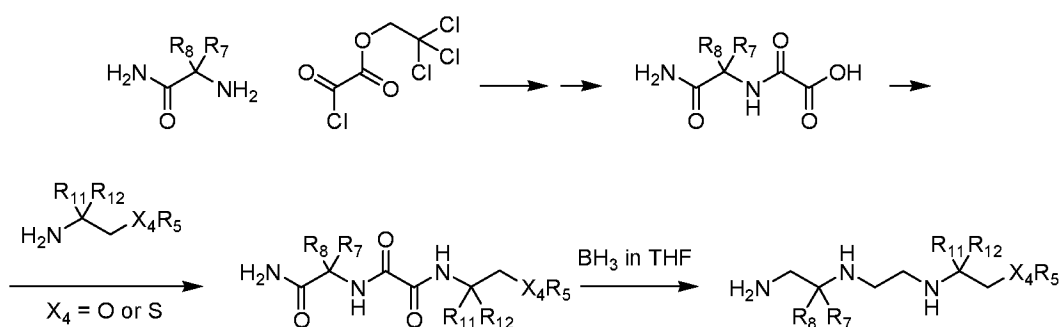
[00131] The incorporation of R1, R2, R5 and R6 can be accomplished with this
25 chemistry by standard procedures.

38



[00132] For the cases where X₄ = S a similar approach using standard peptide synthesis according to the so-called reverse Rink approach as shown above can be used. Coupling with Fmoc protected natural and un-natural amino acids, which can be conveniently cleaved at the penultimate step of the synthesis, generates a modified tri-peptide C-terminal amide. The incorporation of X₄ = S is achieved by the use of an alpha-substituted carboxylic acid in the last coupling step. This is reduced using Diborane in THF to give the open chain tetra-aza compounds.

[00133] The incorporation of R₁, R₂, R₅ and R₆ can be accomplished with this chemistry by standard procedures.



[00134] The oxalamide approach, shown above, can also lead to successful syntheses of this class of compounds, although the central substituents are always going to be hydrogen or its isotopes with this kind of chemistry. This particular variant makes use of the trichloroethyl ester group to protect one of the carboxylic acid functions of oxalic acid but other protecting groups are also envisaged. Reaction of an amino acid amide derived from a natural or unnatural amino acid with a differentially protected oxalyl mono chloride gives the mono-oxalamide shown which can be reacted under standard peptide coupling conditions with an ethanolamine or ethanethiolamine derivative to give the un-symmetrical bis-oxalamide which can then be reduced with diborane as shown to give the desired tri-aza derivative.

3NX series 2:

when X₁, X₂, and X₄ are N and X₃ is O or S then:

R₄ does not exist, and

R₁, R₂, R₃, R₅, and R₆ are independently chosen from H, CH₃, C2-C10 straight
5 chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di,
tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl
mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused
aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH);

n₁, n₂, and n₃ are independently chosen to be 2 or 3, and each repeat of any of
10 n₁, n₂, and n₃ may be the same as or different than any other repeat; and

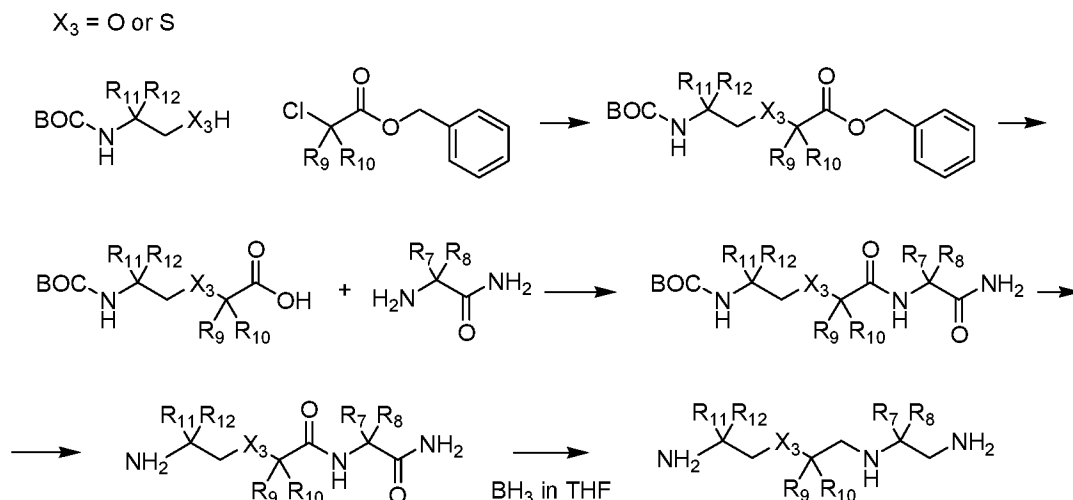
R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10
straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl,
mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-
C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl
15 fused aryl.

[00135] In addition, one or several of R₁, R₂, R₃, R₅, or R₆ may be
functionalized for attachment, for example, to peptides, proteins, polyethylene
glycols and other such chemical entities in order to modify the overall pharmaco-
kinetics, deliverability and/or half lives of the constructs. Examples of such
20 functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10
alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-
protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

[00136] Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be
functionalized for attachment, for example, to peptides, proteins, polyethylene
25 glycols and other such chemical entities in order to modify the overall pharmaco-
kinetics, deliverability and/or half lives of the constructs. Examples of such
functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10
alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-
protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-
30 protein.

Synthesis of examples of the open chain 3NX series 2 of Formula I:

[00137] A different approach can be used for the synthesis of the 3N series 2
class of compounds. The key component is the incorporation in the synthesis of an
appropriately substituted and protected ethanolamine or ethanethiolamine derivative,
35 which is readily available from both natural and un-natural amino acids, as shown
below.



[00138] The BOC protected ethanolamine or ethanethiolamine is reacted with an appropriate benzyl protected α chloroacid. After hydrogenation to deprotect the ester function, standard peptide coupling with a natural or unnatural amino acid amide followed by deprotection and reduction with diborane in THF gives the open chain tri-aza compounds. If hydrogenation is not compatible with other functionality in the molecule then alternative combinations of protecting groups can be used such as trichloroethyloxy carbonyl and t-butyl.

[00139] The incorporation of R1, R2, R5 and R6 can be accomplished with this chemistry by standard procedures.

2N2X series 1:

when X_2 and X_3 are N and X_1 and X_4 are O or S then:

R_1 and R_6 do not exist;

R_2 , R_3 , R_4 , and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO}(\text{OH})_2$, $\text{CH}_2\text{P}(\text{CH}_3)\text{O}(\text{OH})$;

n_1 , n_2 , and n_3 are independently chosen to be 2 or 3, and each repeat of any of n_1 , n_2 , and n_3 may be the same as or different than any other repeat; and

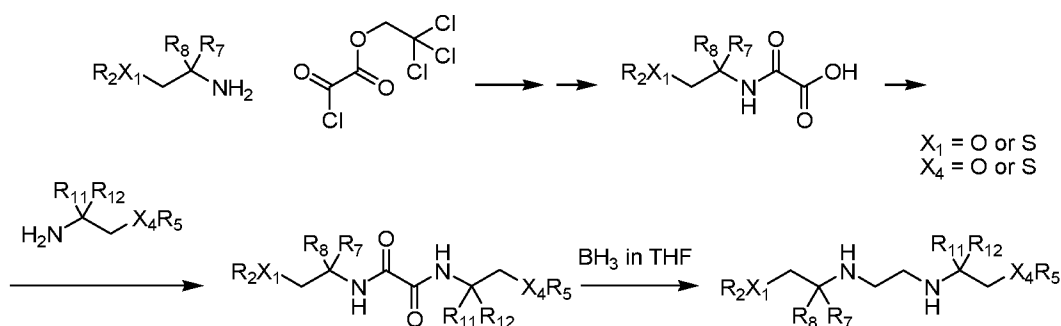
R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-

C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

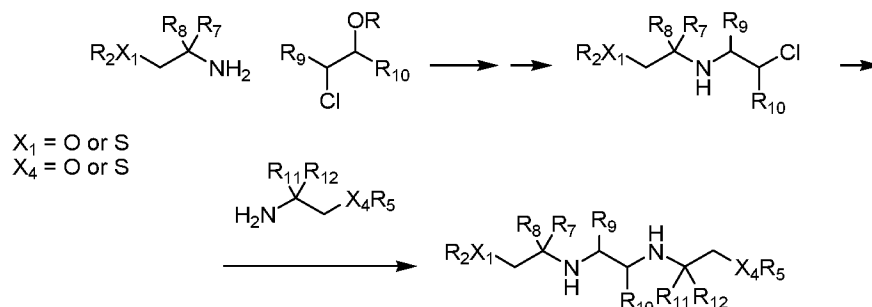
[00140] In addition, one or several of R2, R3, R4, or R5 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00141] Furthermore one or several of R7, R8, R9, R10, R11, or R12 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

Synthesis of examples of the open chain 2N2X series 1 of Formula I:



[00142] The oxalamide approach, shown above, can lead to successful syntheses of this class of compounds. This particular variant makes use of the trichloroethyl ester group to protect one of the carboxylic acid functions of oxalic acid but other protecting groups are also envisaged. Reaction of an aminoalcohol or aminothiols derivative readily available from a natural or unnatural amino acid with a differentially protected oxalyl mono chloride gives the mono-oxalamide shown which can be reacted under standard peptide coupling condition to give the un-symmetrical bis-oxalamide which can then be reduced with diborane to give the desired tetra-aza derivative.



[00143] A variant of the dichloroethane approach, shown above, can also lead to successful syntheses of this class of compounds. Reaction of an aminoalcohol or aminothiols derivative readily available from a natural or unnatural amino acid with an O-protected 1-chloro, 2-hydroxy ethane derivative followed by deprotection and substitution with chloride gives the mono-chloro compound shown which can be further reacted with an appropriate aminoalcohol or aminothiols derivative readily available from a natural or unnatural amino acid to give the un-symmetrical desired product.

2N2X series 2:

when X_1 and X_3 are N and X_2 and X_4 are O or S then:

R_3 and R_6 do not exist;

R_1 , R_2 , R_4 , and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO}(\text{OH})_2$, $\text{CH}_2\text{P}(\text{CH}_3)\text{O}(\text{OH})$;

n_1 , n_2 , and n_3 are independently chosen to be 2 or 3, and each repeat of any of n_1 , n_2 , and n_3 may be the same as or different than any other repeat; and

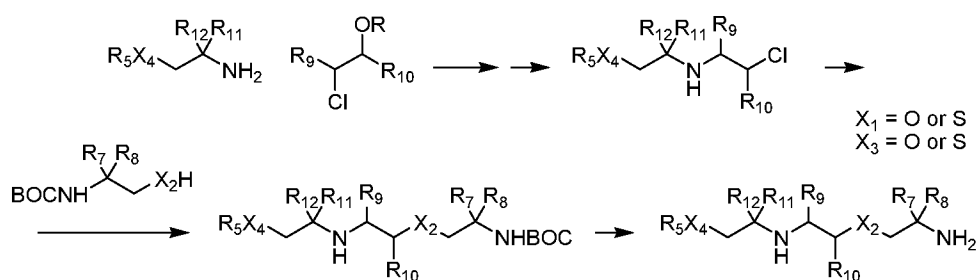
R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

[00144] In addition, one or several of R_1 , R_2 , R_4 , or R_5 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-

PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

[00145] Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , or R_{12} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

Synthesis of the open chain 2N2X series 2 of Formula I:



[00146] A variant of the dichloroethane approach, shown above, can lead to successful syntheses of this class of compounds. Reaction of an aminoalcohol or aminothiols derivative readily available from a natural or unnatural amino acid with an O-protected 1-chloro, 2-hydroxy ethane derivative followed by deprotection and substitution with chloride gives the mono-chloro compound shown which can be further reacted with an appropriately protected aminoalcohol or aminothiols derivative, readily available from a natural or unnatural amino acid, to give the unsymmetrical desired product after de-protection.

2N2X series 3:

when X_1 and X_2 are N and X_3 and X_4 are O or S then:

R_4 and R_6 do not exist;

R_1 , R_2 , R_3 , and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO}(\text{OH})_2$, $\text{CH}_2\text{P}(\text{CH}_3)\text{O}(\text{OH})$;

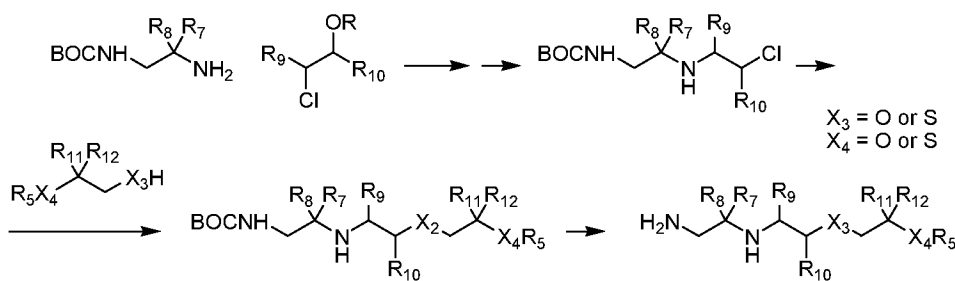
n1, n2, and n3 are independently chosen to be 2 or 3, and each repeat of any of n1, n2, and n3 may be the same as or different than any other repeat; and

R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C₂-C₁₀ straight chain or branched alkyl, C₃-C₁₀ cycloalkyl, C₁-C₆ alkyl C₃-C₁₀ cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C₁-C₆ alkyl aryl, C₁-C₆ alkyl mono, di, tri, tetra and penta substituted aryl, C₁-C₅ alkyl heteroaryl, C₁-C₆ alkyl fused aryl.

[00147] In addition, one or several of R₁, R₂, R₃, or R₅ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C₁-C₁₀ alkyl-CO-peptide, C₁-C₁₀ alkyl-CO-protein, C₁-C₁₀ alkyl-CO-PEG, C₁-C₁₀ alkyl-NH-peptide, C₁-C₁₀ alkyl-NH-protein, C₁-C₁₀ alkyl-NH-CO-PEG, C₁-C₁₀ alkyl-S-peptide, and C₁-C₁₀ alkyl-S-protein.

[00148] Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C₁-C₁₀ alkyl-CO-peptide, C₁-C₁₀ alkyl-CO-protein, C₁-C₁₀ alkyl-CO-PEG, C₁-C₁₀ alkyl-NH-peptide, C₁-C₁₀ alkyl-NH-protein, C₁-C₁₀ alkyl-NH-CO-PEG, C₁-C₁₀ alkyl-S-peptide, and C₁-C₁₀ alkyl-S-protein.

Synthesis of the open chain 2N2X series 3:



[00149] A variant of the dichloroethane approach, shown above, can lead to successful syntheses of this class of compounds. Reaction of a monoprotected ethylene diamine derivative, readily available from a natural or unnatural amino acid with an O-protected 1-chloro, 2-hydroxy ethane derivative followed by deprotection and substitution with chloride gives the mono-chloro compound shown which can be further reacted with an appropriately protected bis-alcohol or bis thiol derivative,

readily available from a natural or unnatural amino acid, to give the un-symmetrical desired product after de-protection.

2N2X series 4:

when X₁ and X₄ are N and X₂ and X₃ are O or S then:

5 R₃ and R₄ do not exist;

R₁, R₂, R₅ and R₆ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl,
10 CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH);

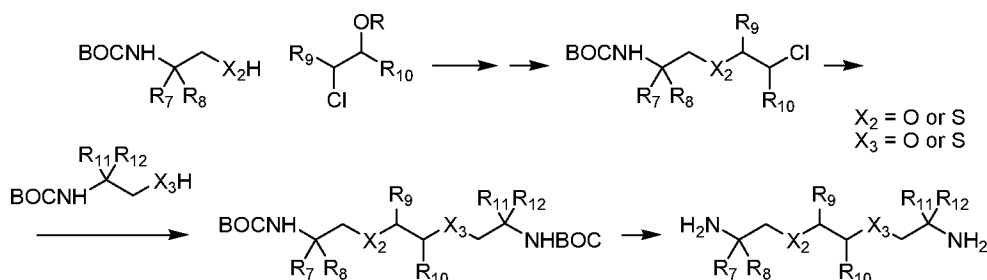
n₁, n₂, and n₃ are independently chosen to be 2 or 3, and each repeat of any of n₁, n₂, and n₃ may be the same as or different than any other repeat; and

R₇, R₈, R₉, R₁₀, R₁₁, and R₁₂ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.
15

[00150] In addition, one or several of R₁, R₂, R₅, or R₆ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.
20

[00151] Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, or R₁₂ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.
30

Synthesis of the open chain 2N2X series 4 of Formula I:



[00152] A variant of the dichloroethane approach, shown above, can lead to successful syntheses of this class of compounds. Reaction of an appropriately protected bis-alcohol or bis thiol derivative, readily available from a natural or unnatural amino acid, with an O-protected 1-chloro, 2-hydroxy ethane derivative followed by deprotection and substitution with chloride gives the mono-chloro compound shown which can be further reacted with an appropriately protected bis-alcohol or bis thiol derivative, readily available from a natural or unnatural amino acid, to give the un-symmetrical desired product after de-protection.

For the Tetra-heteroatom cyclic series:

One of R_1 and R_2 (if R_1 does not exist) and one of R_5 (if R_6 does not exist) and R_6 are joined together to form the bridging group $(CR_{13}R_{14})_{n4}$;

X_1 , X_2 , X_3 , and X_4 are independently chosen from the atoms N, S or O such that:

4N macrocyclic series:

when X_1 , X_2 , X_3 , and X_4 are N then:

R_2 , R_3 , R_4 , and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO}(\text{OH})_2$, $\text{CH}_2\text{P}(\text{CH}_3)\text{O}(\text{OH})$;

n_1 , n_2 , n_3 , and n_4 are independently chosen to be 2 or 3, and each repeat of any of n_1 , n_2 , n_3 and n_4 may be the same as or different than any other repeat; and

R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , R_{13} and R_{14} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

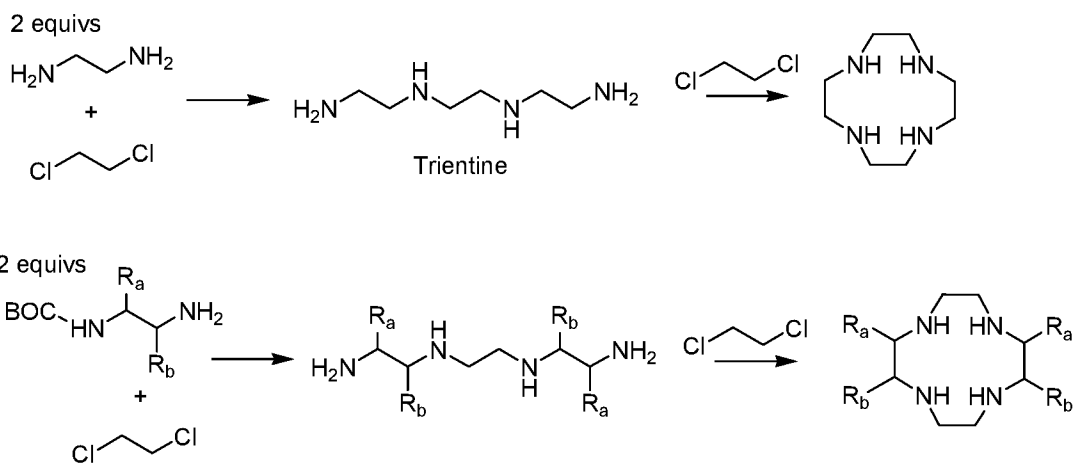
[00153] In addition, one or several of R_2 , R_3 , R_4 , or R_5 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such

chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

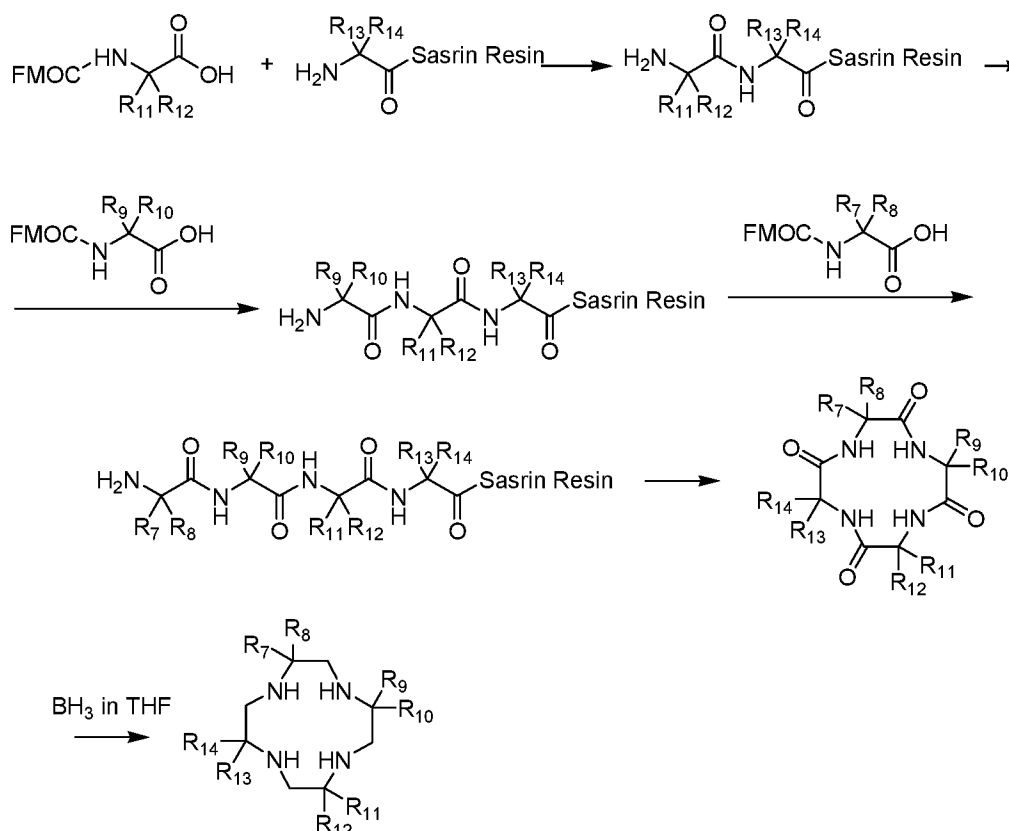
[00154] Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , R_{13} or R_{14} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

Synthesis of examples of the macrocyclic 4N series of Formula I:

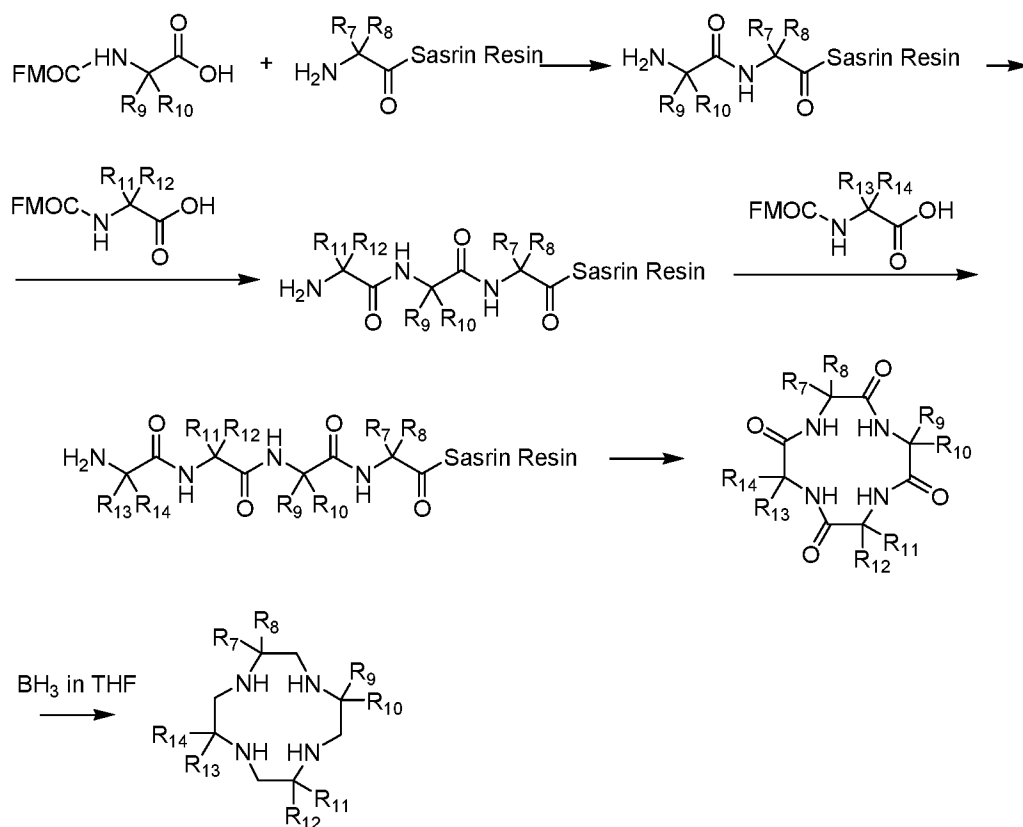
[00155] Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give triethylenetetramine directly (1). Possible side products from this synthesis include the 12N4 macrocycle shown below, which could also be synthesized directly from Triethylenetetramine by reaction with a further equivalent of 1,2-dichloro ethane under appropriately dilute concentrations to provide the 12N4 macrocycle shown. Modification of this procedure by using starting materials with appropriate R_a and R_b (where R_a , R_b correspond to R_7 , R_8 or R_{11} , R_{12}) groups would lead to symmetrically substituted 12N4 macrocycle examples as shown below:



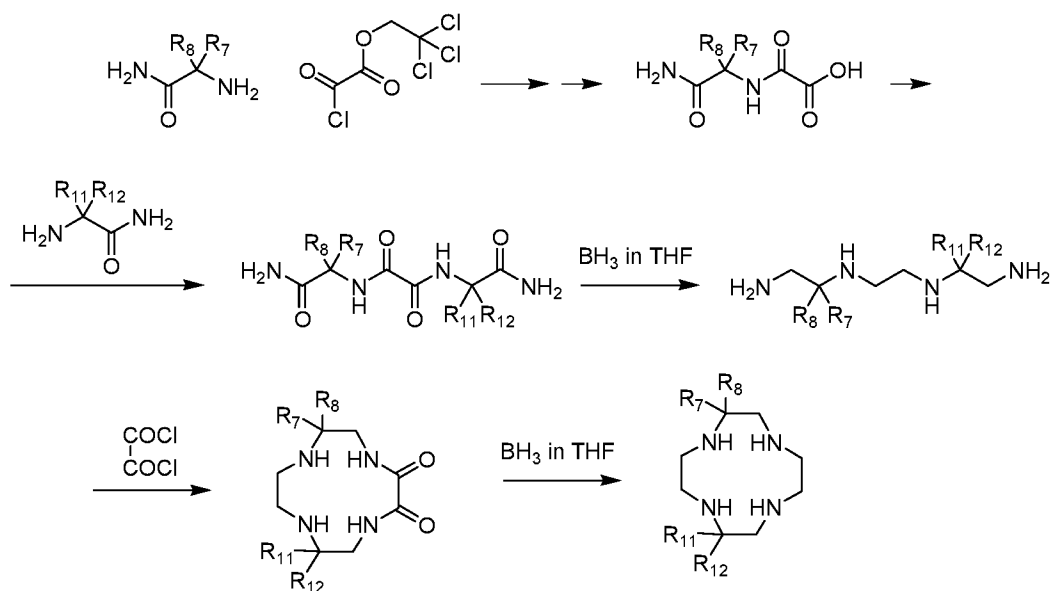
[00156] The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the tetra-aza series. In order to obtain the un-symmetrically substituted derivatives a variant of some chemistry described by Meares et al (3) should be used. Standard peptide synthesis using the Merrifield approach or the SASRIN resin along with Fmoc protected natural and un-natural amino acids which can be conveniently cleaved at a later step of the synthesis generates a fully protected tetra-peptide C-terminal SASRIN derivative. Cleavage of the N terminal Fmoc protecting group followed by direct cyclization upon concomitant cleavage from the resin gives the macrocyclic tetrapeptide. This is reduced using Diborane in THF to give the 12N4 series of compounds as shown below:



[00157] The incorporation of R_1 , R_2 , R_5 and R_6 can be accomplished with this chemistry by standard procedures.



[00158] The reverse Merrifield/SASRIN approach, shown above, also leads to this class of tetra-aza derivatives and may be useful in cases where peptide coupling of a sterically hindered amino acid requires multiple coupling attempts in order to achieve success in the initial Merrifield approach.



[00159] The oxalamide approach, shown above, also can lead to successful syntheses of this class of compounds. This particular variant makes use of the trichloroethyl ester group to protect one of the carboxylic acid functions of oxalic acid but other protecting groups are also envisaged. Reaction of an amino acid amide derived from a natural or unnatural amino acid with a differentially protected oxalyl mono chloride gives the mono-oxalamide shown which can be reacted under standard peptide coupling condition to give the un-symmetrical bis-oxalamide which can then be reduced with diborane to give the desired tetra-aza derivative. Further reaction with oxalic acid gives the cyclic derivative, which can then be reduced once again with diborane to give the 12N4 series of compounds.

3NX series:

when X₁, X₂, X₃, are N and X₄ is S or O then:

R₅ does not exist;

R₂, R₃, and R₄ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH);

n₁, n₂, n₃, and n₄ are independently chosen to be 2 or 3, and each repeat of any of n₁, n₂, n₃ and n₄ may be the same as or different than any other repeat; and

R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ are independently chosen from H, CH₃, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-

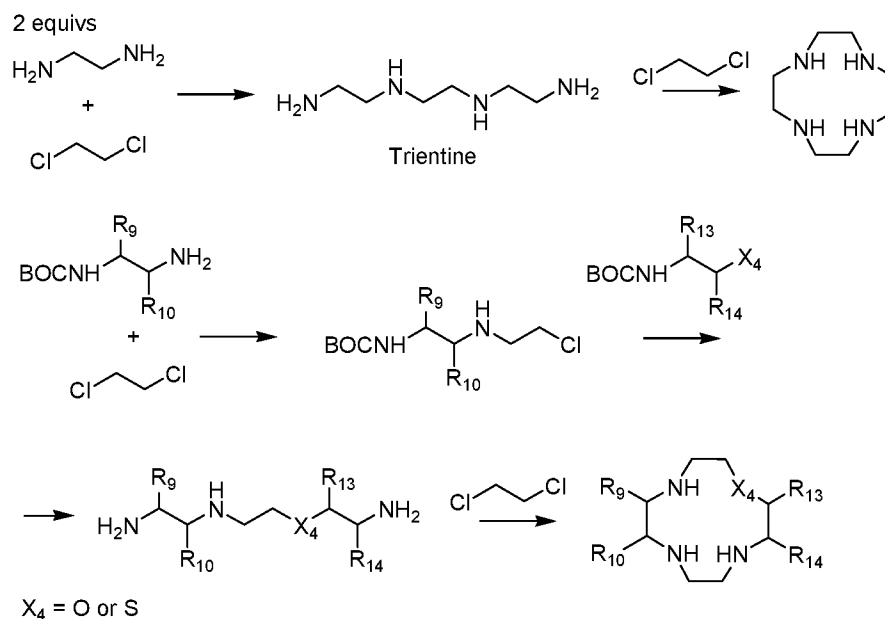
C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

5 **[00160]** In addition, one or several of R₂, R₃ or R₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

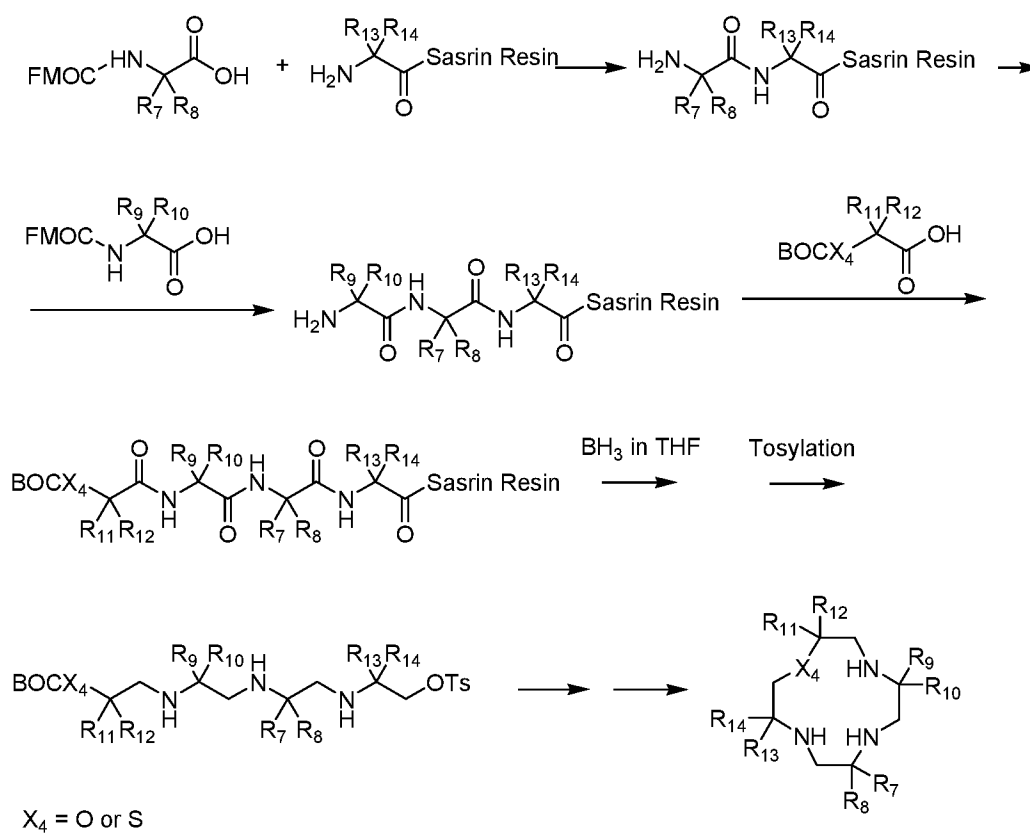
10 **[00161]** Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ or R₁₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10
15 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

Synthesis of examples of the macrocyclic 3NX series of Formula I:

20 **[00162]** Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give triethylenetetramine directly (1). Possible side products from this synthesis include the 12N4 macrocycle shown below, which could also be synthesized directly from Triethylenetetramine by reaction with a further equivalent of 1,2-dichloro ethane under appropriately dilute concentrations to provide the 12N4 macrocycle shown. Modification of this procedure by using starting materials with appropriate R groups leads to symmetrically
25 substituted 12N4 macrocycle examples as shown below:

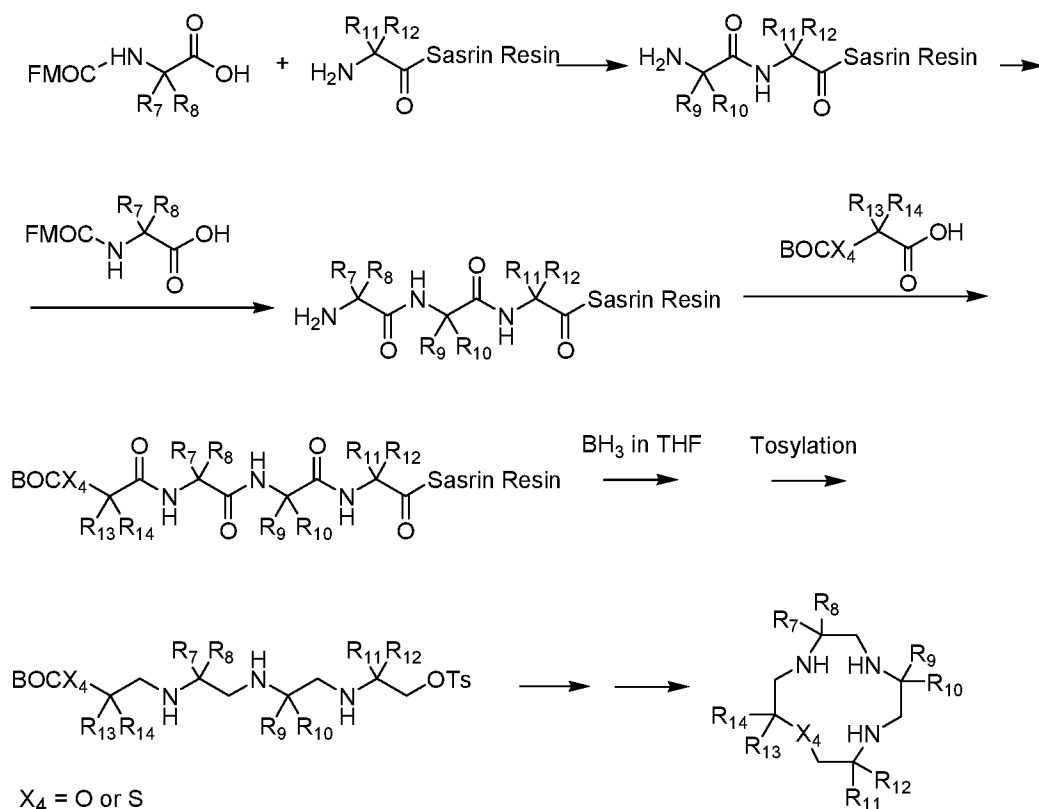


[00163] The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the tri-aza X series. In order to obtain alternative un-symmetrically substituted derivatives a variant of some chemistry described by Meares et al (3) could be used. Standard peptide synthesis using the Merrifield approach or the SASRIN resin along with Fmoc protected natural and un-natural amino acids which can be conveniently cleaved at a later step of the synthesis generates a tri-peptide C-terminal SASRIN derivative which can be further elaborated with an appropriate BOCO or BOCS compound the give the resin bound 3NX compound shown. Reduction with diborane followed by Tosylation would give the 3NX OTosyl linear compound, which, upon deprotection and cyclization would give the desired 3NX macrocycle as shown below:



[00164] The incorporation of R₁, R₂, R₅ and R₆ can be accomplished with this chemistry by standard procedures.

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[00165] The reverse Merrifield/SASRIN approach, shown above, also leads to this class of tetra-aza derivatives and may be useful in cases where peptide coupling of a sterically hindered amino acid requires multiple coupling attempts in order to achieve success in the initial Merrifield approach.

2N2X series 1:

when X_2 and X_3 are N and X_1 and X_4 are O or S then:

R_2 and R_5 do not exist

R_3 and R_4 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO}(\text{OH})_2$, $\text{CH}_2\text{P}(\text{CH}_3)\text{O}(\text{OH})$;

n_1 , n_2 , n_3 , and n_4 are independently chosen to be 2 or 3, and each repeat of any of n_1 , n_2 , n_3 and n_4 may be the same as or different than any other repeat; and

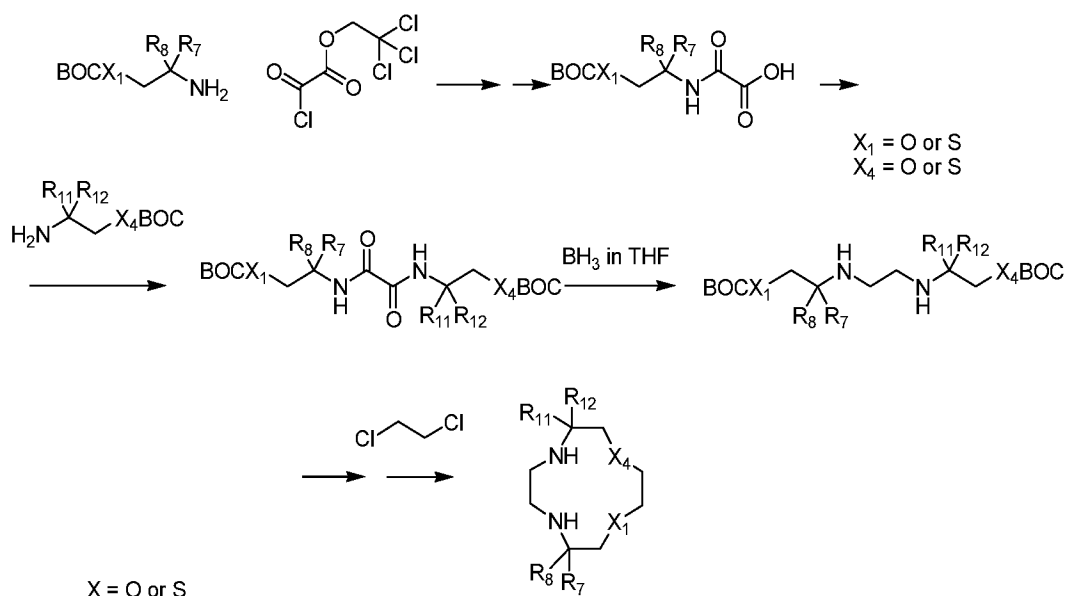
R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , R_{13} and R_{14} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl,

mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl

[00166] In addition, one or both of R_3 , or R_4 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

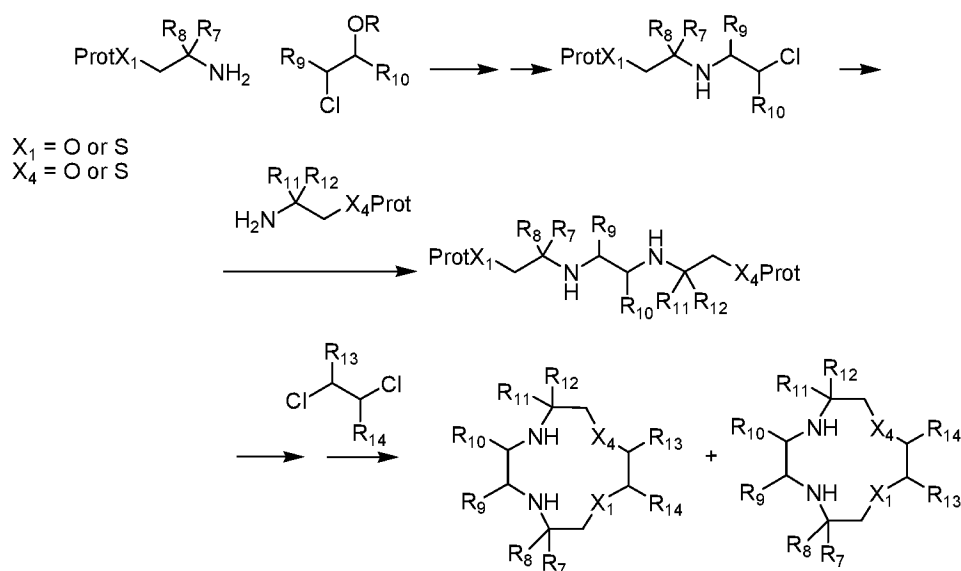
[00167] Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , R_{13} or R_{14} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

Synthesis of examples of the macrocyclic 2N2X series 1 of Formula I:



[00168] The oxalamide approach, shown above, again can lead to successful syntheses of this class of compounds, although the central substituents are always going to be hydrogen or its isotopes with this kind of chemistry. This particular variant makes use of the trichloroethyl ester group to protect one of the carboxylic

acid functions of oxalic acid but other protecting groups are also envisaged. Reaction of an aminoalcohol or aminothioli derivative readily available from a natural or unnatural amino acid with a differentially protected oxalyl mono chloride gives the mono-oxalamide shown which can be reacted under standard peptide coupling condition to give the un-symmetrical bis-oxalamide which can then be reduced with diborane to give the desired di-aza derivative. Deprotection followed by cyclization would give the 12N2X2 analogs.



[00169] A variant of the dichloroethane approach, shown above, can also lead to successful syntheses of this class of compounds. Reaction of an aminoalcohol or aminothioli derivative readily available from a natural or unnatural amino acid with an O-protected 1-chloro, 2-hydroxy ethane derivative followed by deprotection and substitution with chloride gives the mono-chloro compound shown which can be further reacted with an appropriate aminoalcohol or aminothioli derivative readily available from a natural or unnatural amino acid to give the un-symmetrical product shown. Deprotection followed by cyclization with a dichloroethane derivative would give a mixture of the two position isomers shown.

2N2X series 2:

when X_1 and X_3 are N and X_2 and X_4 are O or S then:

R_3 and R_5 do not exist

R_2 and R_4 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di,

tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃O)(OH);

n₁, n₂, n₃, and n₄ are independently chosen to be 2 or 3, and each repeat of any of n₁, n₂, n₃ and n₄ may be the same as or different than any other repeat; and

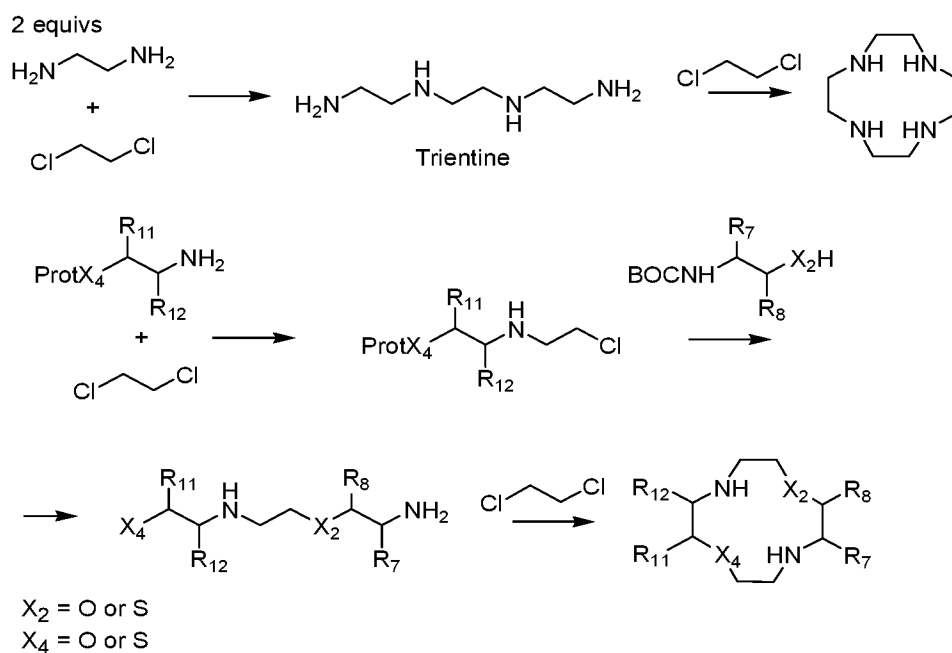
5 R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ are independently chosen from H, CH₃, C₂-C₁₀ straight chain or branched alkyl, C₃-C₁₀ cycloalkyl, C₁-C₆ alkyl C₃-C₁₀ cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C₁-C₆ alkyl aryl, C₁-C₆ alkyl mono, di, tri, tetra and penta substituted aryl, C₁-C₅ alkyl heteroaryl, C₁-C₆ alkyl fused aryl.

10 **[00170]** In addition, one or both of R₂, or R₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C₁-C₁₀ alkyl-CO-peptide, C₁-C₁₀ alkyl-CO-protein, C₁-C₁₀ alkyl-CO-
15 PEG, C₁-C₁₀ alkyl-NH-peptide, C₁-C₁₀ alkyl-NH-protein, C₁-C₁₀ alkyl-NH-CO-PEG, C₁-C₁₀ alkyl-S-peptide, C₁-C₁₀ alkyl-S-protein.

[00171] Furthermore one or several of R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃ or R₁₄ may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-
20 kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C₁-C₁₀ alkyl-CO-peptide, C₁-C₁₀ alkyl-CO-protein, C₁-C₁₀ alkyl-CO-PEG, C₁-C₁₀ alkyl-NH-peptide, C₁-C₁₀ alkyl-NH-protein, C₁-C₁₀ alkyl-NH-CO-PEG, C₁-C₁₀ alkyl-S-peptide, and C₁-C₁₀ alkyl-S-protein.

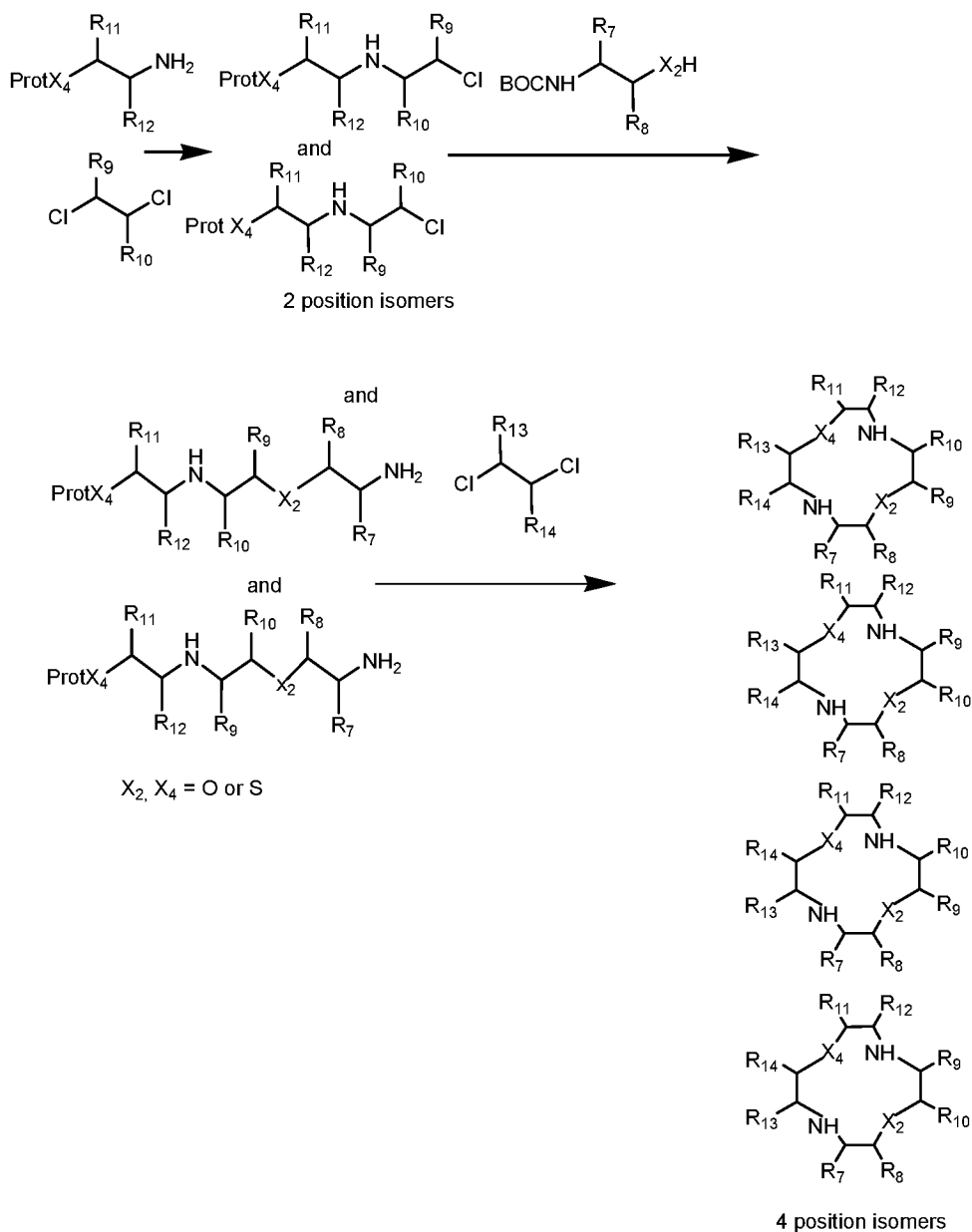
25 **Synthesis of examples of the macrocyclic 2N2X series 2 of Formula I:**

[00172] Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give triethylenetetramine directly (1). Possible side products from this synthesis include the 12N4 macrocycle shown below, which could also be synthesized directly from Triethylenetetramine by
30 reaction with a further equivalent of 1,2-dichloro ethane under appropriately dilute concentrations to provide the 12N4 macrocycle shown. Modification of this procedure by using starting materials with appropriate R groups would lead to symmetrically substituted 12N4 macrocycle examples as shown below:



- 5 **[00173]** The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group and an appropriate O or S protecting group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the di-aza 2X series. A variant of this approach using substituted dichloroethane derivatives could be used to access more complex substitution
- 10 patterns. This would lead to mixtures of position isomers, which can be separated by HPLC.

59

**1N3X series:**

when X_1 is N and X_2, X_3 and X_4 are O or S then:

5

R_3, R_4 and R_5 do not exist;

R_2 is independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO}(\text{OH})_2$, $\text{CH}_2\text{P}(\text{CH}_3)\text{O}(\text{OH})$;

10

n1, n2, n3, and n4 are independently chosen to be 2 or 3, and each repeat of any of n1, n2, n3 and n4 may be the same as or different than any other repeat; and

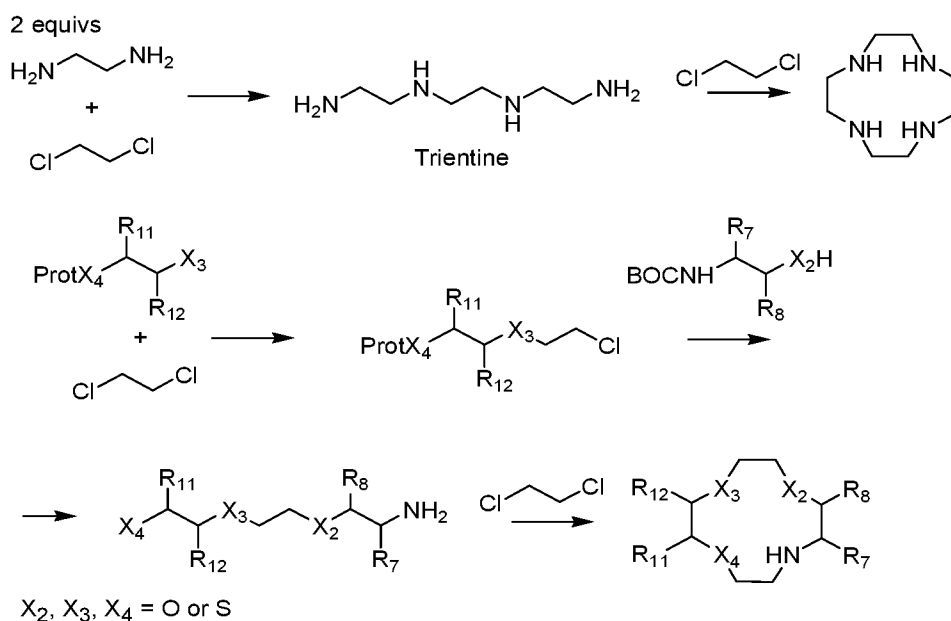
R7, R8, R9, R10, R11, R12, R13 and R14 are independently chosen from H, CH3, C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

[00174] In addition, R2 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00175] Furthermore one or several of R7, R8, R9, R10, R11, R12, R13 or R14 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

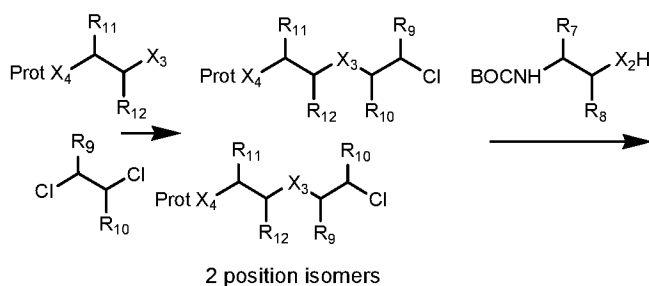
Synthesis of examples of the macrocyclic 1N3X series of Formula I:

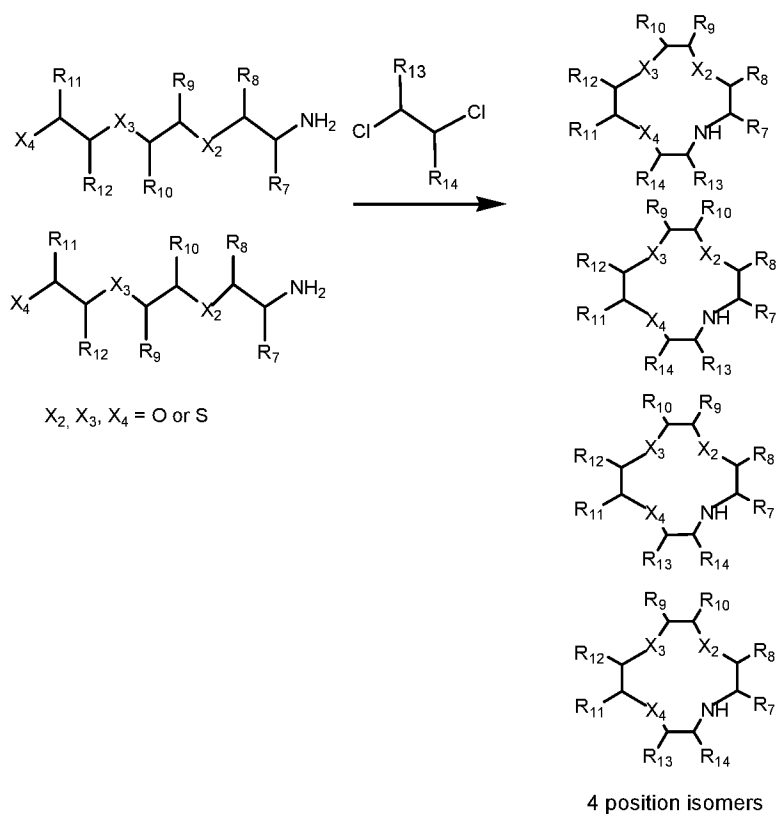
[00176] Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give triethylenetetramine directly (1). Possible side products from this synthesis include the 12N4 macrocycle shown below, which could also be synthesized directly from Triethylenetetramine by reaction with a further equivalent of 1,2-dichloro ethane under appropriately dilute concentrations to provide the 12N4 macrocycle shown. Modification of this procedure by using starting materials with appropriate R groups would lead to substituted 12NX3 macrocycle examples as shown below:



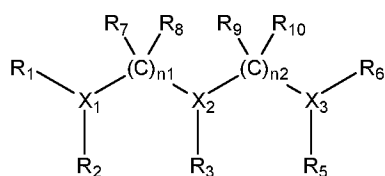
5 **[00177]** The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group and an appropriate O or S protecting group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the mono-aza 3X series. A variant of this approach using substituted dichloroethane derivatives could be used to access more complex substitution patterns. This would lead to mixtures of position isomers, which can be separated by

10 HPLC.





For the tri-heteroatom acyclic examples of Formula II:



5

$X_1, X_2,$ and X_3 are independently chosen from the atoms N, S or O such that:

3N series:

when $X_1, X_2,$ and X_3 are N then:

$R_1, R_2, R_3, R_4,$ and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO}(\text{OH})_2$, $\text{CH}_2\text{P}(\text{CH}_3)\text{O}(\text{OH})$;

n_1 and n_2 are independently chosen to be 2 or 3, and each repeat of any of n_1 and n_2 may be the same as or different than any other repeat; and

15

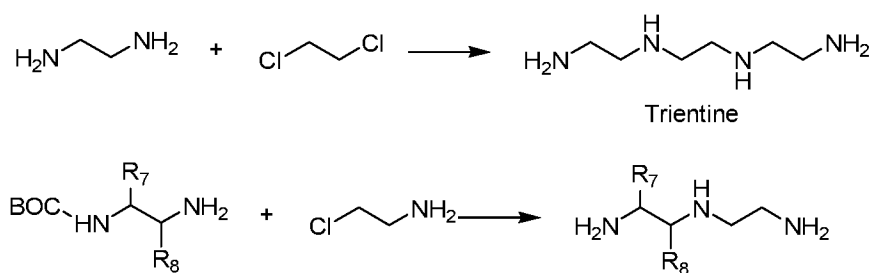
R_7 , R_8 , R_9 , and R_{10} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

5 **[00178]** In addition, one or several of R_1 , R_2 , R_3 , R_5 or R_6 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

15 **[00179]** Furthermore one or several of R_7 , R_8 , R_9 , or R_{10} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

Synthesis of the open chain 3N series of Formula II:

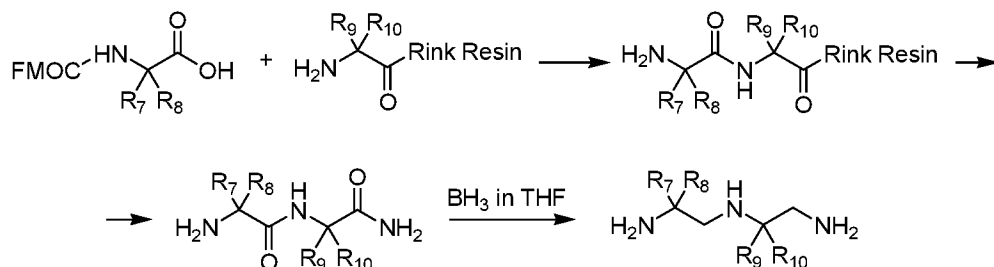
20 **[00180]** As mentioned above Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give Triethylenetetramine directly (1). A variant of this procedure by using starting materials with appropriate R groups and 1-amino,2-chloro ethane would lead to some open chain 3N examples as shown below:



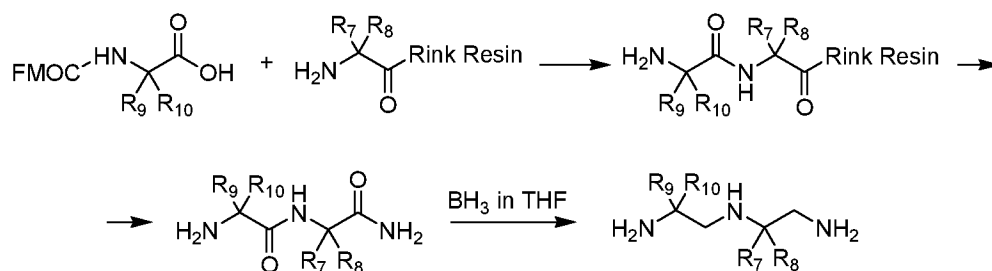
25

30 **[00181]** The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the tri-aza series. In order to obtain the un-symmetrically substituted derivatives a variant of some chemistry

described by Meares et al (2) could be used. Standard peptide synthesis using the Rink resin along with Fmoc protected natural and un-natural amino acids which can be conveniently cleaved at the penultimate step of the synthesis generates a di-peptide C-terminal amide. This can be reduced using Diborane in THF to give the open chain tri-aza compounds as shown below:



[00182] The reverse Rink approach may also be useful where peptide coupling is slowed for a particular substitution pattern as shown below. Again the incorporation of R₁, R₂, R₅ and R₆ can be accomplished with this chemistry by standard procedures:



2NX series 1:

when X₁ and X₃ are N and X₂ is S or O then:

R₃ does not exist

R₁, R₂, R₅, and R₆ are independently chosen from H, CH₃, C₂-C₁₀ straight chain or branched alkyl, C₃-C₁₀ cycloalkyl, C₁-C₆ alkyl C₃-C₁₀ cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C₁-C₆ alkyl aryl, C₁-C₆ alkyl mono, di, tri, tetra and penta substituted aryl, C₁-C₅ alkyl heteroaryl, C₁-C₆ alkyl fused aryl, CH₂COOH, CH₂SO₃H, CH₂PO(OH)₂, CH₂P(CH₃)O(OH);

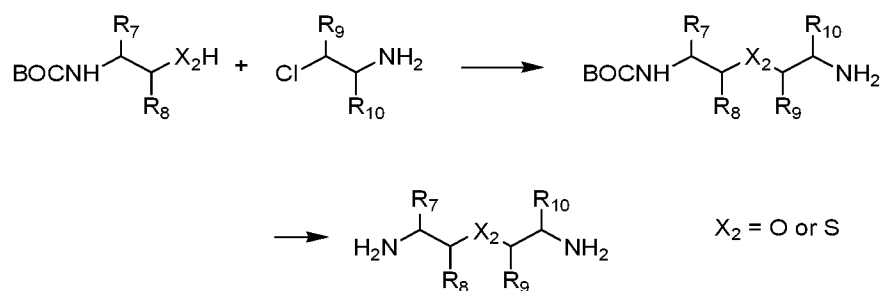
n₁ and n₂ are independently chosen to be 2 or 3, and each repeat of any of n₁ and n₂ may be the same as or different than any other repeat; and

R_7 , R_8 , R_9 , and R_{10} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl

5 **[00183]** In addition, one or several of R_1 , R_2 , R_5 or R_6 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

15 **[00184]** Furthermore one or several of R_7 , R_8 , R_9 , or R_{10} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

20 **Synthesis of the open chain 2NX series 1 of Formula II:**



25 **[00185]** The synthesis of the 2NX series 1 compounds can be readily achieved as shown above. The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown above. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the tri-aza X series.

2NX series 2

when X_1 and X_2 are N and X_3 is O or S then:

R_5 does not exist;

R_1 , R_2 , R_3 and R_6 are independently chosen from H, CH_3 , C2-C10 straight chain or
 5 branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra
 and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di,
 tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl,
 CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$;

n_1 and n_2 are independently chosen to be 2 or 3, and each repeat of any of n_1
 10 and n_2 may be the same as or different than any other repeat; and

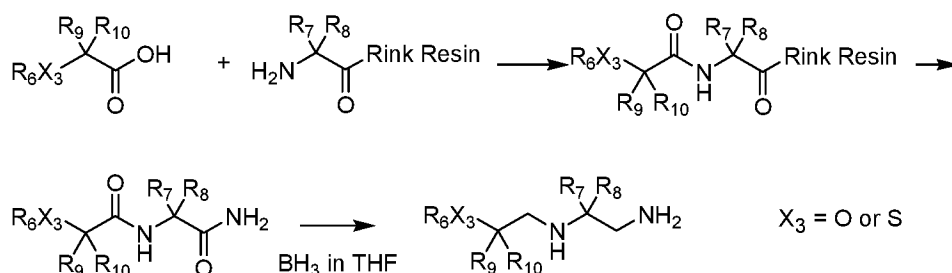
R_7 , R_8 , R_9 , and R_{10} are independently chosen from H, CH_3 , C2-C10 straight chain
 or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri,
 tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono,
 di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

15 **[00186]** In addition, one or several of R_1 , R_2 , R_5 , or R_6 may be functionalized for
 attachment, for example, to peptides, proteins, polyethylene glycols and other such
 chemical entities in order to modify the overall pharmaco-kinetics, deliverability
 and/or half lives of the constructs. Examples of such functionalization include but
 are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-
 20 CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-
 PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00187] Furthermore one or several of R_7 , R_8 , R_9 , or R_{10} may be functionalized for
 attachment, for example, to peptides, proteins, polyethylene glycols and other such
 chemical entities in order to modify the overall pharmaco-kinetics, deliverability
 25 and/or half lives of the constructs. Examples of such functionalization include but
 are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-
 CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-
 PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

Synthesis of the open chain 2NX series 2 of Formula II:

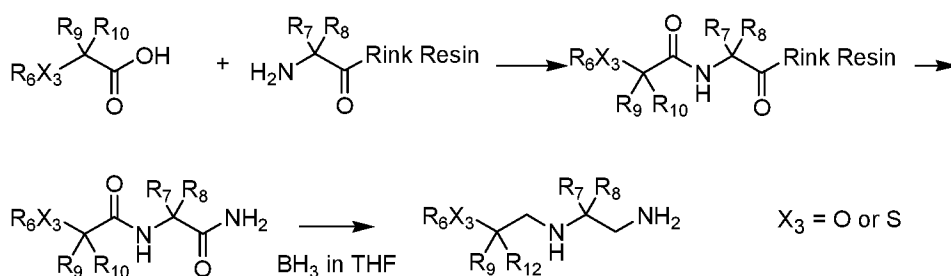
30



[00188] For the cases where $X_3 = O$ or S a similar approach using standard peptide synthesis according to the Rink approach as shown above can be used. Coupling of a suitably protected alpha thio or hydroxy carboxylic acid with a Rink resin amino acid derivative followed by cleavage gives the desired linear di-amide, which can be reduced with Diborane in THF to give the open chain 2NX compounds.

[00189] The incorporation of R_1 , R_2 , R_5 and R_6 can be accomplished with this chemistry by standard procedures.

[00190] The reverse Rink version is also feasible and again the incorporation of R_1 , R_2 , R_5 and R_6 can be accomplished with this chemistry by standard procedures.



Tri-heteroatom cyclic series of Formula II:

R_1 and R_6 form a bridging group $(CR_{11}R_{12})_{n3}$; and

X_1 , X_2 , and X_3 are independently chosen from the atoms N, S or O such that:

3N series:

when X_1 , X_2 and X_3 are N then:

R_2 , R_3 , and R_5 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$;

n_1 , n_2 , and n_3 are independently chosen to be 2 or 3, and each repeat of any of n_1 , n_2 and n_3 may be the same as or different than any other repeat; and

R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

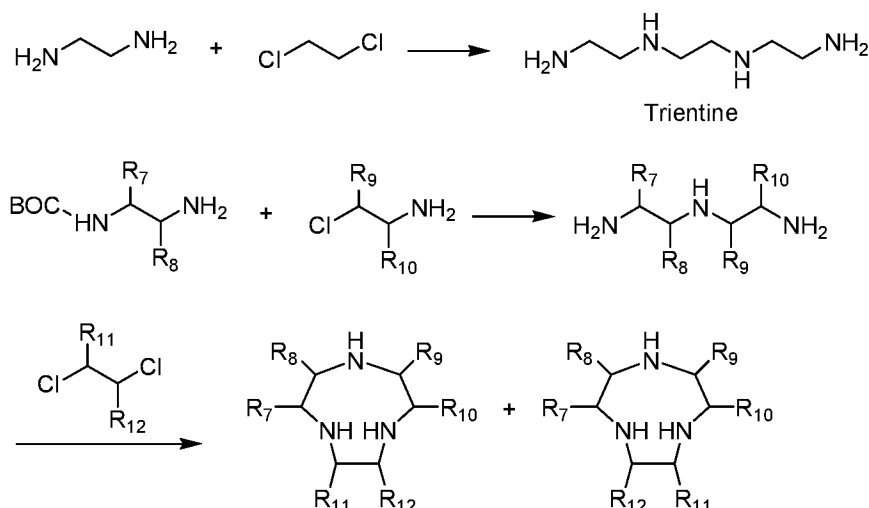
[00191] In addition, one or several of R_2 , R_3 , or R_5 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such

chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00192] Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , or R_{12} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, C1-C10 alkyl-S-protein.

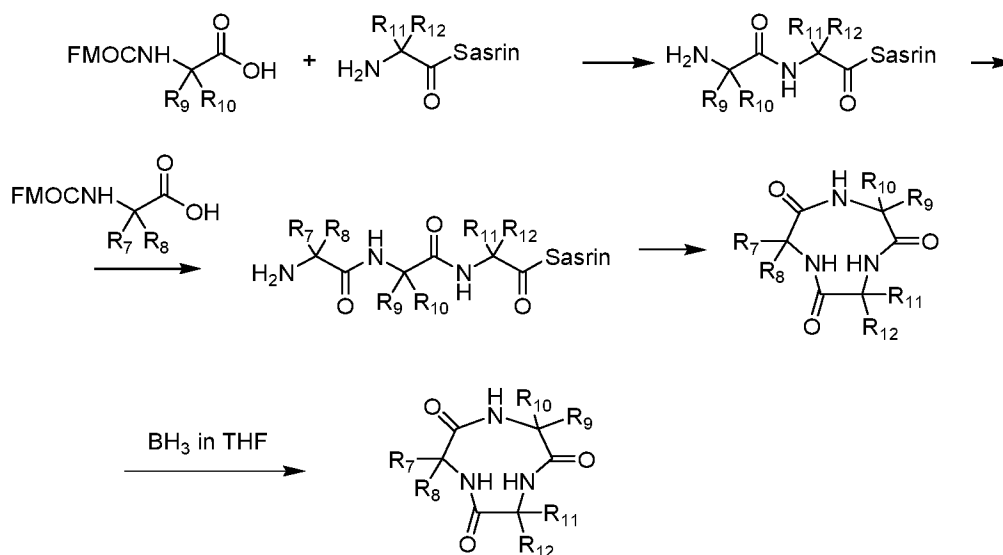
Synthesis of examples of the macrocyclic 3N series of Formula II:

[00193] As mentioned above Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give Triethylenetetramine directly (1). A variant of this procedure by using starting materials with appropriate R groups and 1-amino,2-chloro ethane would lead to open chain 3N examples which could then be cyclized by reaction with an appropriate 1,2 dichloroethane derivative as shown below:



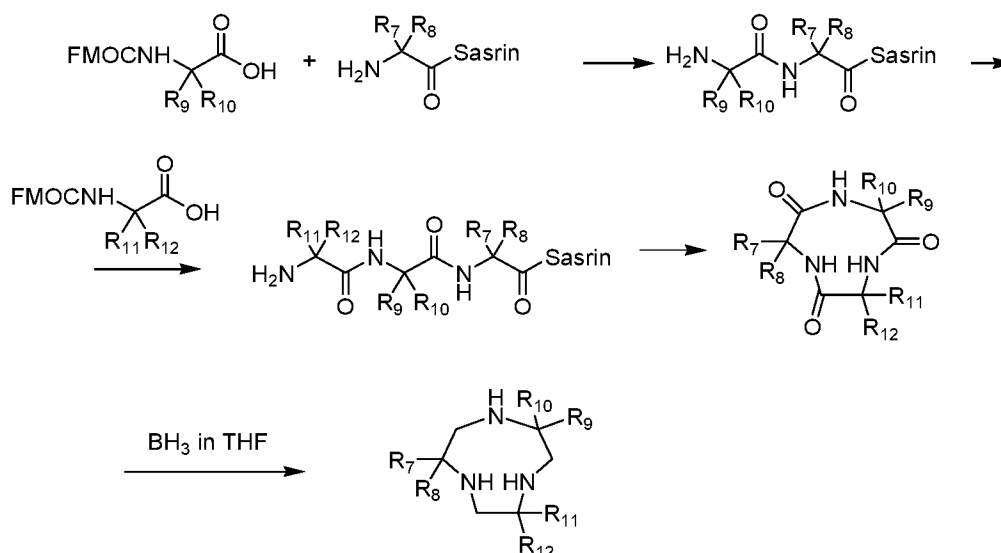
[00194] The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the macrocyclic tri-aza series. In

order to obtain the un-symmetrically substituted derivatives a variant of some chemistry described by Meares et al (3) could be used. Standard peptide synthesis using the Merrifield approach/SASRIN resin along with Fmoc protected natural and un-natural amino acids which can be conveniently cleaved at the penultimate step of the synthesis generates a tri-peptide attached to resin via it's C-terminus. This can be cyclized during concomitant cleavage from the resin followed by reduction using Diborane in THF to give the cyclic tri-aza compounds as shown below:



[00195] The incorporation of R₁, R₂, and R₅ can be accomplished with this chemistry by standard procedures.

[00196] The reverse Rink approach may also be useful where peptide coupling is slowed for a particular substitution pattern as shown below. Again the incorporation of R₁, R₂, R₅ and R₆ can be accomplished with this chemistry by standard procedures:

**2NX series:**

when X_1 and X_2 are N and X_3 is S or O then:

R_5 does not exist;

5 R_2 and R_3 are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , $\text{CH}_2\text{SO}_3\text{H}$, $\text{CH}_2\text{PO(OH)}_2$, $\text{CH}_2\text{P(CH}_3\text{)O(OH)}$;

10 n_1 , n_2 , and n_3 are independently chosen to be 2 or 3, and each repeat of any of n_1 , n_2 and n_3 may be the same as or different than any other repeat; and

R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-
15 C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

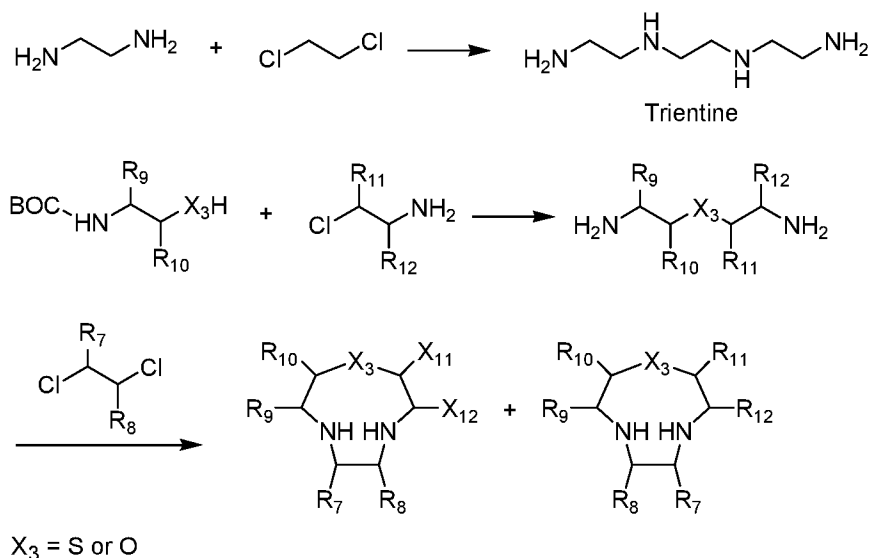
[00197] In addition, one or both of R_2 or R_3 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability
20 and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00198] Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , or R_{12} may be
25 functionalized for attachment, for example, to peptides, proteins, polyethylene

glycols and other such chemical entities in order to modify the overall pharmacokinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

Synthesis of examples of the macrocyclic 2NX series of Formula II:

[00199] As mentioned above Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give Triethylenetetramine directly (1). A variant of this procedure by using starting materials with appropriate R groups and 1-amino,2-chloro ethane would lead to open chain 2NX examples which could then be cyclized by reaction with an appropriate 1,2 dichloroethane derivative as shown below:

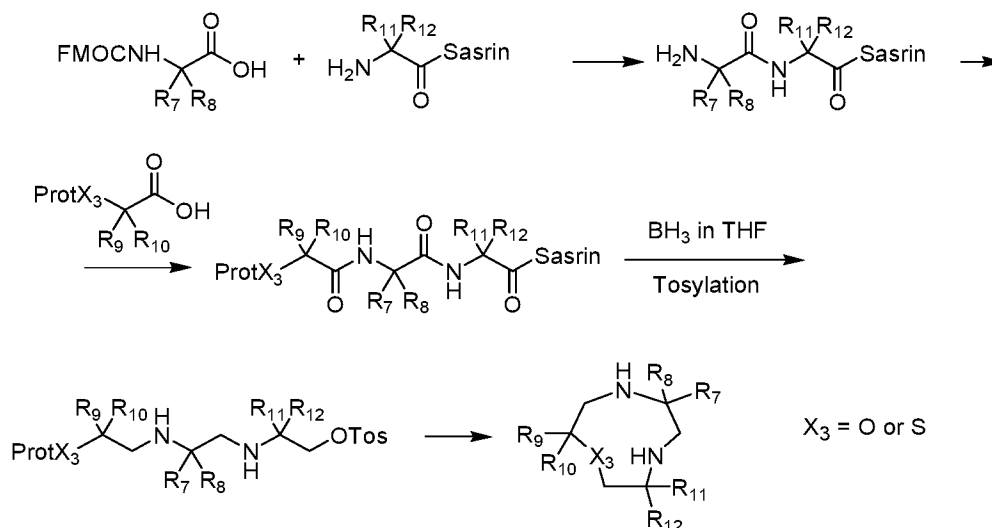


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[00200] The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the macrocyclic di-aza X series. In order to obtain the un-symmetrically substituted derivatives a variant of some chemistry described by Meares et al (3) could be used. Standard peptide synthesis using the Merrifield approach/SASRIN resin along with FMOC protected natural and un-natural amino acids which can be conveniently cleaved at the penultimate step of

20

the synthesis generates a tri-peptide attached to resin via its C-terminus. This can be cyclized during concomitant cleavage from the resin followed by reduction using Diborane in THF to give the cyclic tri-aza compounds as shown below:

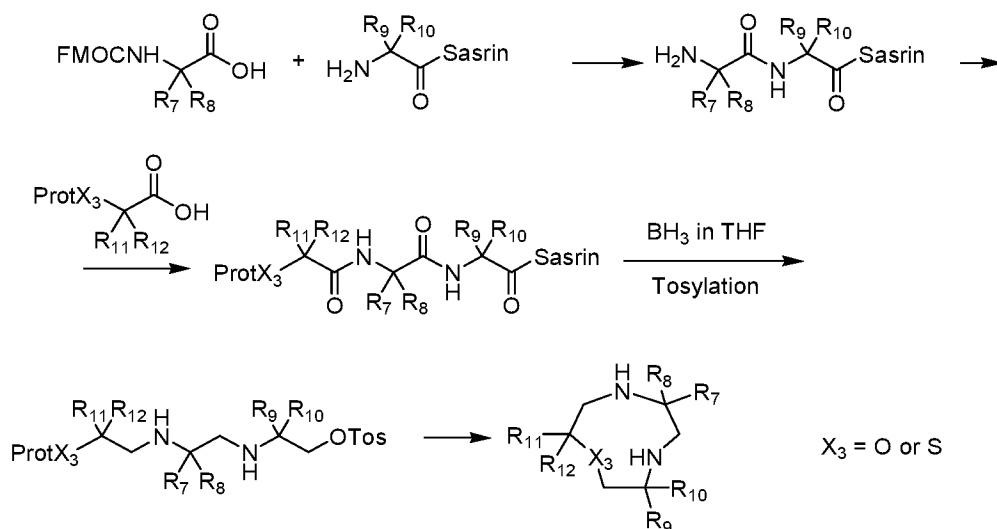


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[00201] The incorporation of R_1 , and R_2 can be accomplished with this chemistry by standard procedures.

[00202] The reverse Rink approach may also be useful where peptide coupling is slowed for a particular substitution pattern as shown below. Again the incorporation of R_1 , and R_2 can be accomplished with this chemistry by standard procedures:

10



1N2X series:

when X_1 is N and X_2 and X_3 are O or S then:

R_3 and R_5 do not exist;

R_2 is independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl, CH_2COOH , CH_2SO_3H , $CH_2PO(OH)_2$, $CH_2P(CH_3)O(OH)$;

n_1 , n_2 , and n_3 are independently chosen to be 2 or 3, and each repeat of any of n_1 , n_2 and n_3 may be the same as or different than any other repeat;

R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{12} are independently chosen from H, CH_3 , C2-C10 straight chain or branched alkyl, C3-C10 cycloalkyl, C1-C6 alkyl C3-C10 cycloalkyl, aryl, mono, di, tri, tetra and penta substituted aryl, heteroaryl, fused aryl, C1-C6 alkyl aryl, C1-C6 alkyl mono, di, tri, tetra and penta substituted aryl, C1-C5 alkyl heteroaryl, C1-C6 alkyl fused aryl.

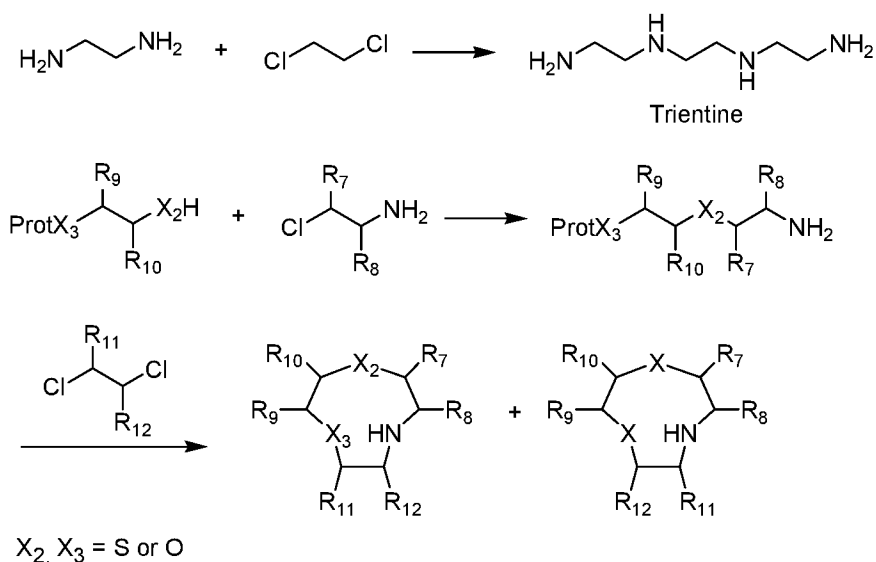
[00203] In addition, R_2 may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

[00204] Furthermore one or several of R_7 , R_8 , R_9 , R_{10} , R_{11} , or R_{12} may be functionalized for attachment, for example, to peptides, proteins, polyethylene glycols and other such chemical entities in order to modify the overall pharmaco-kinetics, deliverability and/or half lives of the constructs. Examples of such functionalization include but are not limited to C1-C10 alkyl-CO-peptide, C1-C10 alkyl-CO-protein, C1-C10 alkyl-CO-PEG, C1-C10 alkyl-NH-peptide, C1-C10 alkyl-NH-protein, C1-C10 alkyl-NH-CO-PEG, C1-C10 alkyl-S-peptide, and C1-C10 alkyl-S-protein.

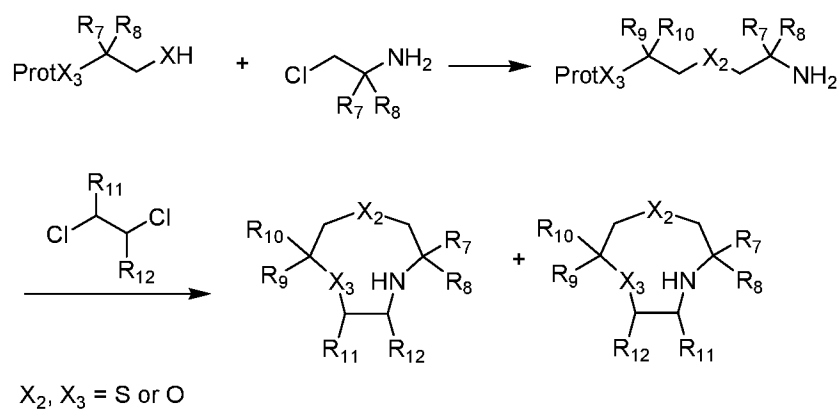
Synthesis of examples of the macrocyclic 1N2X series of Formula II:

[00205] As mentioned above Triethylenetetramine itself has been synthesized by reaction of 2 equivalents of ethylene diamine with 1,2-dichloro ethane to give Triethylenetetramine directly (1). A variant of this procedure by using starting materials with appropriate R groups and 1-amino,2-chloro ethane would lead to open chain 1N2X examples which could then be cyclized by reaction with an appropriate 1,2 dichloroethane derivative as shown below:

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- 5 **[00206]** The judicious use of protecting group chemistry such as the widely used BOC (t-butyloxycarbonyl) group allows the chemistry to be directed specifically towards the substitution pattern shown. Other approaches such as via the chemistry of ethyleneimine (2) may also lead to a subset of the macrocyclic aza di-X series. In order to obtain the un-symmetrically substituted derivatives a variant of some chemistry above could be used:



10

- [00207]** The incorporation of R₁ and R₂ can be accomplished with this chemistry by standard procedures.

[00208] Methods of preparing triethylenetetramines particularly suited for use as described herein are presented in U.S. Patent No. 7,582,796 (Jonas et al., issued 1 September 2009), herein incorporated by reference in its entirety.

EXAMPLES

5 EXAMPLE 1

[00209] This example investigates metabolic perturbations in various brain regions of AD sufferers.

2. Methods

Acquisition of human brains

10 [00210] Whole brains from patients and matched controls were obtained from the New Zealand Neurological Foundation Human Brain Bank, in the Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand. All procedures in this study were approved by the University of
15 Auckland Human Participants Ethics Committee with informed consent from all families. The quality of AD brain tissue acquired by the Human Brain Bank was uniformly high and only those with very short post-mortem delays (~4-13 h) were used for the current study.

Sampling of human-brain tissue

20 [00211] After receipt into the Human Brain Bank, brains were dissected under the supervision of neuroanatomists, to ensure accurate identification of each of the seven brain regions targeted in this study. The brain regions included three regions known to undergo severe neuronal damage in AD (the hippocampus (HP), entorhinal cortex (ENT) and middle-temporal gyrus (MTG)) three regions known to be less severely
25 affected (the sensory cortex (SCx), motor cortex (MCx) and cingulate gyrus (CG)), and one region known to be relatively spared (the cerebellum (CB)). Tissue samples of 50 ± 5 mg were dissected from each region and stored at -80°C until analysis.

Diagnosis and severity of AD

30 [00212] All AD patients had clinical dementia, whereas controls did not. Control brains were selected from the Human Brain Bank by matching for age, sex and post-mortem delay as shown in Table 1. A consultant neuropathologist diagnosed or

excluded AD by applying the Consortium to Establish a Registry for AD (CERAD) criteria.

Table 1: Group characteristics of brains used in study

Variable	Control	AD
Number	9	9
Age ($\pm$ SD)	70.1 ($\pm$ 6.7)	70.3 ($\pm$ 7.1)
Male sex, n (%)	5 (55.6)	5 (55.6)
Post-mortem delay (h, range)	9 (5.5-13.0)	7 (4.0-12.0)
Brain weight (g, range)	1260 (1094-1461)	1062* (831-1355)

* $P=0.005$ compared with Control; all other differences were non-significant.

5

Tissue extraction

[00213] Brain tissues placed in "Safe-Lok" microfuge tubes (Eppendorf AG; Hamburg, Germany) were held at -80°C until extraction. They then underwent a Folch-style extraction using a TissueLyser batch bead homogeniser (Qiagen; Manchester, UK). Briefly, each sample containing 50 ± 5 mg of brain tissue was extracted in 0.8 ml 50:50 (v/v) methanol:chloroform, to which a solution of the labelled internal standards in methanol had been added to achieve a final concentration of 0.016 mg/ml of each internal standard in the extraction solvent (kept at -20°C until used). A set of seven isotopically-labelled standards (Citric acid- d_4 , $^{13}\text{C}_6$ -D-fructose, Ltryptophan- d_5 , L-alanine- d_7 , stearic acid- d_{35} , benzoic acid- d_5 , and leucine- d_{10}) purchased from Cambridge Isotopes Inc (Tewksbury, MA) were used in this study. Extraction was performed for 10 min at 25 Hz with a single 3-mm tungsten carbide bead per tube. Samples corresponding to the same brain region were handled as single separate batches for this and all subsequent procedures. Separation of phases was achieved by addition of 0.4-ml water followed by vortex-mixing (10-15 s) and centrifugation (2,400 g, 15 min). After separation, tissue debris lay at the interface between the lower (non-polar, chloroform) phase and the upper (polar, methanol:water) phase containing the target molecules for the current study. For each batch, extraction blanks were prepared by processing tubes containing solvent and bead, but no tissue sample. This procedure produced clean polar extracts with low levels of lipid and protein content, which are known to otherwise cause response-instability in the GC-MS method.

Sample preparation

[00214] Chloroform in extraction tubes was removed using a 500- μ l HPLC syringe (Sigma Aldrich, MO, USA). Tubes were then centrifuged (16,000 g, 15 min) to encourage tissue debris to form a coherent pellet. From the methanol:water supernatant, 200- μ l aliquots were transferred to pre-labelled tubes containing 600 μ l of methanol, to precipitate residual protein. A quality-control (QC) pool was made by combining 200- μ l aliquots from each extraction. The pooled samples were gently mixed and 200- μ l portions dispensed into tubes containing 600- μ l methanol. Both sample and QC tubes were centrifuged (16,000 g, 15 min) and 750- μ l aliquots were transferred to a final set of pre-labelled tubes which were processed to dryness in a Speedvac centrifugal concentrator ($\sim$ 30 $^{\circ}$ C, 16-18 h (Savant; SPD331DDA, Thermo Scientific)). Dried residues were held in sealed tubes at 4 $^{\circ}$ C for up to one week (shown to be stable for eight weeks for serum previously stored) until derivatization for GC-MS analysis.

GC-MS Analysis

[00215] Methyloxime/trimethylsilyl derivatives were prepared by a two-step procedure. GC-MS analysis was performed using an MPS2 autosampler (Gerstel; Mülheim an der Ruhr, Germany), a 7890A Gas Chromatograph with Split/Splitless inlet (Agilent; Santa Clara, CA, USA), and a Pegasus HT time-of-flight mass spectrometer (LECO; Stockport, UK).

[00216] Gas chromatography was performed using an Agilent/J&W DB-17MS column (30 m x 0.25 mm x 0.25 μ m; Agilent: #122-4732) with a 3-m deactivated Fused Silica retention gap (0.25 mm; Agilent: #No 160-2256-10), and helium carrier gas (1.4 ml/min, constant flow mode). 1- μ l sample injections were made in Pulse Splitless mode at an inlet temperature of 270 $^{\circ}$ C, using an "empty, hot-needle" technique. The initial column temperature (50 $^{\circ}$ C) was held for 6 min and then increased to 300 $^{\circ}$ C at 10 $^{\circ}$ C /min and held for a further 4 min. This resulted in a total cycle time of 42 min between injections. After an initial 450-s solvent delay (to allow solvent and reagents to elute without damaging the detector), mass spectral data were acquired at 10 spectra/s for the range 45-800 Da, detecting a range of amino acids, sugars, sugar alcohols and organic acids as their TMS derivatives. Standard 70-eV electron energy was employed, at a source temperature of 220 $^{\circ}$ C.

[00217] The study was performed in a series of single-batch experiments, where each specific brain region constituted a batch. Within each batch, individual cases and controls were randomized, and run in a sequence interleaved with injections of

the pooled QC samples (one per four study samples) and extraction blanks (two per batch). A lead-in sequence of six QC injections at the start of each batch was used to condition the chromatographic system. Extraction blanks were inspected visually to confirm absence of carryover, but not included in subsequent data analysis.

5 **Data reduction**

[00218] Data were prepared using the 'Reference Compare' method within ChromaTOF 4.5 (LECO).

[00219] Briefly, the software was used to perform a global peak deconvolution of parameters to compile a list of nominated 'metabolites', and search mass-spectral libraries to generate putative identities. Databases we employed were: the NIST Mass Spectral Reference Library (NIST08/2008; National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health Spectral Library; NIST, Gaithersburg, MD, USA); the Golm Metabolome Database (Max Planck Institute of Molecular Plant Physiology, Potsdam- Golm, Germany); and an in-house library developed at the University of Manchester. Chromatographic retention-time data were available from reference standard compounds for a subset of the identities. Within this subset, matching of both mass spectra and expected retention time(s) was interpreted to constitute a definitive (D) molecular identification. Matching of mass spectra and retention time with reported data was interpreted as confident (C) identification. Matching of mass spectra only was interpreted as a putative (P) identification. From the list of nominations, we compiled reference tables comprising expected mass spectra and retention-time windows. These were then applied as target lists of features to be searched across all the study samples. As the pooled QC samples should contain all metabolite features encountered in the experiment, these were suitable candidates for compilation of reference tables. To provide a robust reference table, the initial list of nominations was edited to remove ambiguous and low quality spectra prior to application. Global deconvolution was performed on several (3-4) pooled QC injections across the entire experiment to improve identifications. By displaying these overlaid while editing the list, reproducible spectra were more readily distinguished from lower quality candidates. The same target list was used for all brain regions.

Metabolite abundance reporting

[00220] In order to use the edited reference table as a reporting tool, appropriate parameters such as mass spectral match thresholds and tolerable retention-time deviations (6 sec) were specified, and the table initialized using a pooled QC sample

to provide reference m/z peak areas. Improved reproducibility was achieved by the use of internal standard ratios rather than raw peak areas.

[00221] The most suitable standard was assigned to each metabolite by determining which internal standard yielded the lowest variance for a given metabolite across all the QC injections.

[00222] The resulting data for each experiment were compiled into a matrix of metabolite-intensity data, which was merged with experimental metadata for visualization and statistical analysis. Although the automated procedure was highly reliable (estimated return of correct peak areas for >95% of features measured), data sets were also curated manually to remove possible integration errors which were mostly associated with metabolites showing non-ideal peak shape.

Statistics

[00223] The merged metadata were used for data analysis. A principal-components analysis (PCA) was performed for visualization to confirm overall data integrity, using SIMCA-P software (UMetrics AB, Umeå, Sweden). Calculation of relative fold-change and statistical analysis were performed in log space using multiple t-tests (GraphPad Prism 6). Data were considered for multiple comparison analysis applying an FDR (10%) correction. The fold-changes were converted to linear space for presentation and metabolites identified in ≥ 5 samples in each group were reported.

3. Results

[00224] There was about 16% median brain weight decline in AD; median (range) brain weight was 1,062 g (831-1,355) in AD and 1,260 g (1,094-1,461; $P < 0.005$) in controls (see Table 1).

[00225] PCA of GC-MS data revealed: 1) excellent class separation in all brain regions between AD and control samples; 2) greater biological than technical variation; and 3) absence of run-order effects in this study (data not shown). One control sample clustered more closely to the AD samples. The brain from which this sample originated had the lowest brain-weight (1,094 g) among the controls and was assessed as Braak stage II by neuropathological examination. On this basis, this control sample was reclassified as a case of preclinical AD case and has been excluded from the subsequent analysis for reasons of clarity.

[00226] 69 metabolite features were categorised per brain region. The metabolites for which an altered abundance in one or more regions of the AD was observed are shown in Table 2. Numbers indicate fold-change (AD/controls). Changes with $P < 0.05$ (10% FDR) were considered significant and were shown in bold italic font. 55 features were shown to change in at least one brain region (FDR corrected multiple t-test ($p < 0.05$)), with individual regions showing between 16 and 33 metabolites identified as significantly changed.

[00227] Metabolites for which no altered abundance was observed for any brain region are shown in Table 3.

Table 2: Relative fold change in metabolites with altered abundance in the AD brain.

Metabolite	HP	ENT	MTG	SCX	MCX	CG	CB
Glucose and related metabolites & pentose phosphate pathway components							
Glucose (D)	12.3	9.3	16.9	10.8	8.0	9.9	6.8
Glucose-6-phosphate (D)	5.9	4.9	8.5	4.8	6.0	4.8	3.8
Sorbitol (D)	3.4	3.8	3.9	5.0	5.3	5.0	4.6
Fructose (D)	4.6	4.8	7.0	6.8	7.0	7.0	7.1
Fructose-6-phosphate (D)	1.2	0.6	5.3	1.6	2.5	7.7	2.3
Pentonic acid A (P)	1.1	1.3	1.3	1.2	1.4	1.4	1.3
Pentonic acid B (P)	2.1	2.0	1.9	1.7	1.7	1.9	1.7
Arabinose (P)	3.5	NM	3.6	3.5	6.8	3.9	NM
Ribose-5-phosphate (D)	0.8	0.6	0.8	1.1	1.1	1.0	0.9
Erythronic acid (P)	1.6	1.8	1.1	1.2	1.2	1.4	1.5
Alternative fuel source							
Butanediol (D)	4.2	4.3	1.6	4.2	9.3	4.1	4.1
β -Hydroxybutyric acid (D)	1.6	2.5	1.5	3.4	1.8	2.5	1.6
Lactic acid (D)	2.6	0.5	1.3	3.4	7.3	1.7	0.6
2-hydroxy-3-methylbutyric acid (P)	7.7	2.3	2.8	4.3	2.5	4.7	3.4
Threitol (D)	2.0	2.3	1.8	2.4	2.3	2.4	3.2
Xylitol (D)	1.7	1.3	1.2	1.2	1.1	1.4	1.7
Disaccharide (D)	4.8	2.3	3.2	3.6	1.8	1.8	0.9
N-acetylglucosamine (C)	1.2	1.1	1.7	1.4	1.4	1.6	2.8
myo-Inositol (D)	1.1	1.9	1.1	1.2	0.8	0.7	0.9
myo-Inositol-1-phosphate (P)	2.3	2.0	4.1	1.7	2.2	3.9	3.5
Glycerol (D)	0.6	0.7	0.8	0.8	0.7	0.9	0.9

Glycerol-2-phosphate (P)	1.9	1.6	1.5	1.7	1.8	1.6	1.6
Glycerol-3-phosphate (D)	2.3	2.7	1.4	2.5	2.6	1.8	1.6
Glyceric acid (P)	1.3	1.1	2.8	1.5	1.1	2.6	1.8
TCA cycle & urea cycle							
Citric acid (D)	1.7	2.1	1.7	1.9	1.1	1.1	1.1
Malic acid (C)	1.6	1.9	2.4	1	0.8	1.7	0.9
Fumaric acid (C)	1.8	1.3	1.7	1.2	0.8	1.4	1.3
Ornithine (D)	0.6	0.6	1.0	0.7	0.7	0.9	0.3
Urea (D)	6.5	5.6	4.7	4.9	5.0	5.3	4.9
N-acetylglutamic acid (D)	0.4	0.8	0.9	0.8	0.7	0.8	1.0
Creatinine (D)	1.1	1.0	1.5	1.0	1.0	1.2	1.2
Amino acids							
Proline (D)	0.5	0.4	0.5	0.7	0.8	0.8	0.5
Lysine (D)	0.5	0.7	1.1	0.8	1.1	1.1	0.3
Glycine (D)	0.9	0.7	0.8	0.8	0.7	0.9	0.9
Serine (D)	0.7	0.6	1.0	0.7	0.8	1.0	0.7
Threonine (D)	0.8	1.3	1.5	1.1	2.2	1.4	1.7
Cysteine (D)	2.0	1.4	0.7	1.0	1.0	1.4	0.9
beta-Alanine (D)	1.2	1.0	1.3	1.1	1.1	1.3	0.9
Aspartic acid (D)	0.6	0.6	0.8	1.0	0.9	0.9	0.8
N-acetylaspargic acid (D)	0.7	0.8	0.7	1.0	0.9	0.9	1.0
Glutamic acid (P)	1.3	1.0	1.0	1.3	1.3	1.3	2.6
GABA (D)	1.3	0.6	0.5	0.7	0.7	0.9	0.8
4-hydroxybutyric acid (C)	0.6	0.6	1.1	0.8	0.8	0.7	0.7
Phenylalanine (D)	1.3	1.2	2.1	1.6	1.8	2.0	1.3
Tryptophan (D)	2.5	2.2	4.7	1.8	3.2	4.0	1.1
Nucleosides							
Adenine (D)	1.0	1.0	0.9	1.0	0.9	0.9	1.7
Uracil (C)	0.6	0.5	0.6	0.6	0.5	0.6	0.7
Adenosine-5-monophosphate (P)	1.6	2.1	1.9	1.4	NM	2.4	1.3
Guanosine (D)	0.7	0.8	2.8	0.9	NM	2.5	1.6
Hypoxanthine (D)	0.7	0.7	0.7	0.7	0.7	0.8	0.7
Miscellaneous							
Ethanolamine (D)	0.6	0.4	0.5	0.5	0.5	0.6	0.6
Methyl-phosphate (C)	0.7	0.4	0.4	0.7	0.6	0.5	0.8
Phosphoric acid (D)	1.2	1.1	0.5	0.8	0.7	0.6	0.9
2-Hydroxyglutaric acid (D)	1.9	2.2	1.9	1.6	1.6	1.8	1.4

Ascorbic acid (P)	2.0	1.7	1.1	1.8	2.2	1.6	1.5
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Abbreviations: D, definitive; C, confident; P, putative. Changed with $P < 0.05$ were considered significant and are shown in bold italic font.

Table 3: Relative fold change in metabolites with no statistically significant change in abundance in the AD brain.

Metabolite	HP	ENT	MTG	SCX	MCX	CG	CB
Hydroxylamine (P)	1.0	1.1	0.8	1.0	1.2	0.9	1.2
Alanine (D)	0.8	0.7	1.3	0.6	3.4	1.0	1.2
Pyruvic acid (D)	1.3	1.6	1.5	0.8	0.5	1.0	0.8
Valine (D)	1.6	0.9	2.8	1.0	1.0	1.5	0.5
Leucine (D)	0.9	0.9	1.4	1.0	1.0	1.4	0.8
Isoleucine (D)	0.8	0.8	1.3	0.8	1.0	0.7	1.0
Succinic acid (C)	1.3	0.6	1.1	1.2	1.3	1.2	1.2
Methionine (D)	1.0	0.9	1.3	0.9	1.0	1.4	0.7
Ribitol (P)	0.3	1.0	2.0	0.4	1.2	1.1	1.0
Pyroglutamic acid (D)	1.2	1.2	1.1	1.3	1.5	1.2	1.1
Mannitol (D)	1.1	1.2	1.3	1.3	1.2	1.2	1.8
Scyllo-inositol (D)	0.9	0.9	1.1	0.7	0.7	0.8	0.7
Tyrosine (D)	0.7	0.7	1.2	1.0	1.0	1.2	0.6
Adenosine (D)	2.0	1.3	0.9	1.0	1.0	0.8	1.0

Abbreviations: D, definitive; C, confident; P, putative.

[00228] This example demonstrates alterations in metabolites from several pathways involved in glucose clearance and utilisation.

10 EXAMPLE 2

[00229] This example investigates copper metal levels in various brain regions of AD sufferers.

1. Methods

[00230] Human brains were acquired and sampled as for Example 1. Study group characteristics are shown in Table 4.

[00231] The diagnosis and severity of AD was assessed as for Example 1.

Table 4: Group characteristics of brains used in study

Variable	Control	AD
Number	13	9
Age ($\pm$ SD)	70 ($\pm$ 6.3)	70.3 ($\pm$ 7.1)
Male sex, n (%)	7 (53.8)	5 (55.6)
Post-mortem delay (h, range)	12 (5.5-15.0)	7* (4.0-12.0)
Brain weight (g, range)	1260 (1094-1461)	1062* (831-1355)
Wet-wt/dry-wt	5.7 (5.6-5.9)	5.5 (5.4-5.6)
Plaques, n (%)	1 (7.7)	9 (100)**
Tangles, n (%)	1 (7.7)	9 (100)**

* $P=0.005$, ** $P<0.0001$ compared with Control; all other differences were non-significant.

Preparation of brain extracts

5 **[00232]** Cu concentrations were determined on a dry-weight basis. Brain samples of 50 ± 5 mg wet-weight were first dried to constant weight in a centrifugal concentrator (Savant Speedvac; Thermo-Fisher, Waltham, MA). The precise weights of the dried tissue samples were measured to enable normalization of tissue-metal content by dry-weight (Table 1). Samples were digested in 2-mL microcentrifuge tubes (Eppendorf) as described below.

Digestion

15 **[00233]** Tissue was digested using concentrated nitric acid (A509 Trace Metal Grade; Fisher, Loughborough, UK) to which was added 5% (v/v) Agilent Internal Standard mixture (5183-4681; Agilent Technologies, Cheshire, UK). This internally-standardized acid was also used at appropriate dilutions to provide rinse and calibration solutions, at 2% (v/v) final nitric acid concentration.

20 **[00234]** Calibration solutions were produced by appropriate dilutions of Environmental Calibration Standard (Agilent 5183-4688). Acid digestion was carried out using an 'open-vessel' method. Tissue aliquots were briefly centrifuged to ensure that the tissue sat at the bottom of the tube. The tube lids were punctured to prevent pressure build-up, and 0.2 mL standard-containing nitric acid added. Tubes were then inserted into a "Dri-block" heater which was initially at room temperature. Tubes with standard-containing acid but no sample were processed in each batch to provide "digestion" blanks. Temperature was then set to 60°C and the block switched on. After 30 min, the set temperature was increased to 100°C, and digestion continued for a further 210 min. After digestion, the tubes were allowed to cool overnight.

[00235] Aliquots of 100 μL were taken from each digestion and added to 15-mL Falcon tubes (Greiner) containing 5 mL LC-MS grade water, to produce solutions for analysis at a final nitric acid concentration of 2% (v/v).

ICP-MS

5 **[00236]** Cu-metal concentrations were measured using an Agilent 7700x ICP-MS spectrometer equipped with a MicroMist nebulizer (Glass Expansion, Melbourne, Australia) and a Scott double-pass spray chamber. Nickel sample and skimmer cones were used. Sample introduction was performed using an Agilent Integrated autosampler (I-AS). Helium was used as the collision gas. A multi-element method
10 including all elements present in the calibration solution was applied. Calibration solutions were produced by appropriate dilutions of Environmental Calibration Standard (Agilent 5183-4688). Scandium was used as the internal standard. Two collision cell gas modes were applied, Cu metal concentration was analysed in helium mode (5.0 mL min⁻¹ helium). Mode selection followed Agilent recommendations to
15 minimize interference for measured elements by e.g. isobaric cluster ions. Integration time was 0.3 s. For each analytical batch, multi-element calibration was performed using serial dilutions of the calibration standard. An intermediate concentration from this calibration series was used as a periodic quality-control (QC) sample throughout each analytical batch. Instrument and digestion blanks were also
20 interspersed through each set of randomized samples. The detection limit for Cu was determined by comparison of calibration samples and blanks and any samples below this level were eliminated prior to reporting.

[00237] ICP-MS measures the amount of metals such as copper in their elemental state. In contrast, most metals are present in the human body as cations;
25 in the case of copper, the physiological cations copper(I) and copper(II).

Data analysis

[00238] Datasets were exported to Microsoft Excel worksheets and individual values of each sample were normalized by the corresponding sample weight (dry weight). Weight-adjusted datasets were then log-transformed for statistical analysis.
30 Means ($\pm$ 95% CI) of the log-transformed data were calculated and the significance of between-group differences was examined by unpaired *t*-test with Welch's correction to allow for unequal variances and sample sizes. Means ($\pm$ 95% CI) were back-transformed to reflect the actual elemental concentrations of elements. Statistical calculations were performed using GraphPad v6.04 (Prism; La Jolla, CA).

P-values of <0.05 have been considered significant, and those of $0.05 \leq P < 0.10$ have also been tabulated.

2. Results

[00239] The concentration of the essential metal Cu was measured in seven regions of human post-mortem brains from nine AD and 13 control subjects matched for age and sex.

[00240] One control patient also had neuropathological findings consistent with AD (Braak Stage II) and was therefore diagnosed with premanifest disease: this finding is consistent with the known frequency of asymptomatic AD in similarly-aged groups in the study population. Wet-wt/dry-wt ratios did not differ significantly between cases or controls.

[00241] Mean Cu concentrations in control tissue were highest in CB (710 $\mu\text{mol/kg}$ dry-wt) and lowest in ENT (310 $\mu\text{mol/kg}$ dry-wt) as shown in Table 5. Cu levels were significantly decreased compared with corresponding regional-control values in all seven regions of AD-brain, consistent with the presence of pan-cerebral copper deficiency.

Table 5: Copper metal concentration and wet/wt/dry-wt ratios in seven brain regions of AD and control brains.

Brain region	Cu ($\mu\text{mol/kg}$ dry-wt, mean $\pm$ 95% CI)		
	Control	AD	p-value
HP	330 (260-419)	183 (128-261)	0.0066
ENT	309 (281-340)	202 (146-281)	0.018
MTG	356 (300-422)	239 (195-293)	0.0035
SCX	382 (323-451)	268 (218-330)	0.0079
MCX	390 (319-478)	252 (184-347)	0.019
CG	346 (264-454)	198 (141-280)	0.010
CB	708 (617-811)	374 (323-434)	< 0.0001

Reference isotope = ^{63}Cu . Data are means ($\pm$ 95% CI); P-values for significance of between-group differences were calculated by Welch's modified *t*-tests based on measurements from control (n=13) and AD (n=9) brains.

[00242] This example demonstrates pan-cerebral copper-deficiency in seven regions of the AD brain.

EXAMPLE 3

[00243] This example investigates levels of metabolites and trace metals in brain regions known to undergo varying degrees of damage in AD.

1. Methods

5 [00244] Human brains were acquired and sampled as for Example 1. The study group was the same as that for Example 1. No patients had history consistent with type II diabetes.

[00245] The diagnosis and severity of AD was assessed as for Example 1.

Analytical methods

10 [00246] Metabolite levels were compared between cases and controls by GC-MS-based metabolomics in wet-tissue. Copper levels were measured in dry-tissue by inductively-coupled-plasma mass spectrometry (ICP-MS).

Observational case-control study

15 [00247] Patients and controls without cognitive impairment were respectively recruited through memory clinics in Leeds and Dewsbury (England), and the Leeds Family Health Services Authority day hospitals and elderly medicine outpatient clinics in the Leeds area. All were of European Caucasian background and gave written informed consent (consent from relatives of the AD-patients was provided, where appropriate). Diagnosis of probable AD was made in accordance with international
20 diagnostic criteria (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association Work Group: NINCDS-ADRDA). All participants underwent a standardised clinical evaluation: medical history, fasting plasma glucose (FPG) and HbA1c, and cognitive function assessment by Mini-Mental State Examination (MMSE).

25 [00248] AD samples were selected by excluding patients with diagnosed T1D or T2D including those on synthetic insulin. Patients with a previous medical history of other medical conditions were also excluded. Samples were then selected from the whole-study population for whom required measurements (FPG, HbA1c) were available. The resulting 42 AD patients were then age- and gender-matched to 43
30 controls.

2. Results

Glucose, sorbitol and fructose in AD brain regions

[00249] The relative fold-changes in glucose, sorbitol and fructose in seven brain regions is shown in Table 6.

5 **[00250]** Levels of glucose were elevated in all brain regions of AD patients. Levels of glucose tended to be higher in regions of the brain more severely affected by AD such as the middle temporal gyrus.

10 **[00251]** Sorbitol, formed from glucose, is the first metabolite in the polyol pathway, which usually accounts for a few percent at most of glucose utilization under normal conditions. Sorbitol was elevated in all AD brain regions.

[00252] Fructose is the second metabolite in the polyol pathway. Brain fructose levels were elevated in all AD-brain regions.

Table 6: Relative fold change in glucose, sorbitol and fructose in brain regions.

Metabolite	Brain region	Estimate	Lower Bound	Upper Bound	P-value	BH FDR Q-value
Glucose	Cerebellum	5.2	1.4	18.6	0.015	0.015
	Entorhinal cortex	7.4	2.4	23.2	0.0028	0.0041
	Cingulate gyrus	8.0	2.4	26.1	0.0023	0.0037
	Hippocampus	8.9	2.9	27.4	0.0016	0.0030
	Sensory cortex	9.2	3.0	28.8	0.0014	0.0029
	Motor cortex	11.8	3.7	37.7	0.0006	0.0019
	Middle temporal gyrus	16.4	5.2	51.2	0.0002	0.0019
Sorbitol	Cerebellum	3.7	1.6	8.6	0.0036	0.0044
	Entorhinal cortex	4.3	2.0	9.1	0.0006	0.0019
	Cingulate gyrus	3.1	1.5	6.5	0.0048	0.0053
	Hippocampus	4.1	1.9	8.5	0.0009	0.0021
	Sensory cortex	4.1	1.9	8.6	0.0009	0.0021
	Motor cortex	3.0	1.4	6.6	0.0090	0.0094
	Middle temporal gyrus	3.3	1.5	6.9	0.0039	0.0046
Fructose	Cerebellum	5.3	2.0	14.4	0.0018	0.0031
	Entorhinal cortex	5.7	2.4	13.5	0.0004	0.0019
	Cingulate gyrus	3.9	1.7	9.2	0.0030	0.0041
	Hippocampus	5.5	2.3	12.9	0.0005	0.0019

Sensory cortex	5.4	2.4	12.4	0.0004	0.0019
Motor cortex	4.2	1.7	10.2	0.0032	0.0041
Middle temporal gyrus	5.7	2.6	12.9	0.0003	0.0019

Estimates and their lower and upper bounds were derived by Bayesian modelling. Abbreviation: 'BH FDR Q-value' is the Benjamini-Hochberg False-Discovery Rate-adjusted *P*-value.

5 **[00253]** Elevated brain levels of glucose, sorbitol and fructose were present in one control patient, a 76 year-old female, who had no *ante-mortem* clinical evidence for brain disease or dementia, but had premanifest AD characterised by low brain weight (1,094 g) and positive *post-mortem* histology.

Copper levels in AD brain regions

10 **[00254]** Mean ($\pm 95\%$ CI) brain-copper levels summed across all seven regions were 256 (232-279) $\mu\text{mol/dry kg}$ in AD and 406 (363-449) $\mu\text{mol/dry kg}$ in controls ($P=1.6 \times 10^{-8}$): there was thus an overall decrease of $\sim 40\%$ in brain-copper levels in AD. Mean brain-copper levels tended to be lower in AD than controls in all brain regions examined as shown in Figure 1B. In addition, there was moderate evidence for a trend in copper levels to be inversely proportional to tissue-glucose values in AD-patients ($P=0.021$; restricted iterative generalized least squares, RIGLS 23) but not in controls as shown in Figure 1 (A and B).

15 ***Plasma glucose and plasma copper levels in AD patients***

20 **[00255]** In the case control study group, measured cognitive function (MMSE score) in the AD group was significantly lower than that of controls whereas the ApoE4 allele was more prevalent (data not shown). Fasting plasma glucose (FPG) and serum HbA1c levels were equivalent between groups so there was no evidence for elevated rates of undiagnosed T2D or impaired glucose tolerance (IGT)/impaired fasting glucose (IFG) in this group of British patients with sporadic AD (data not shown). FPG and fasting plasma-copper levels did not differ significantly between the AD group and the controls (data not shown).

25 **[00256]** This example demonstrates elevated glucose, fructose, sorbitol and copper levels in the AD brain.

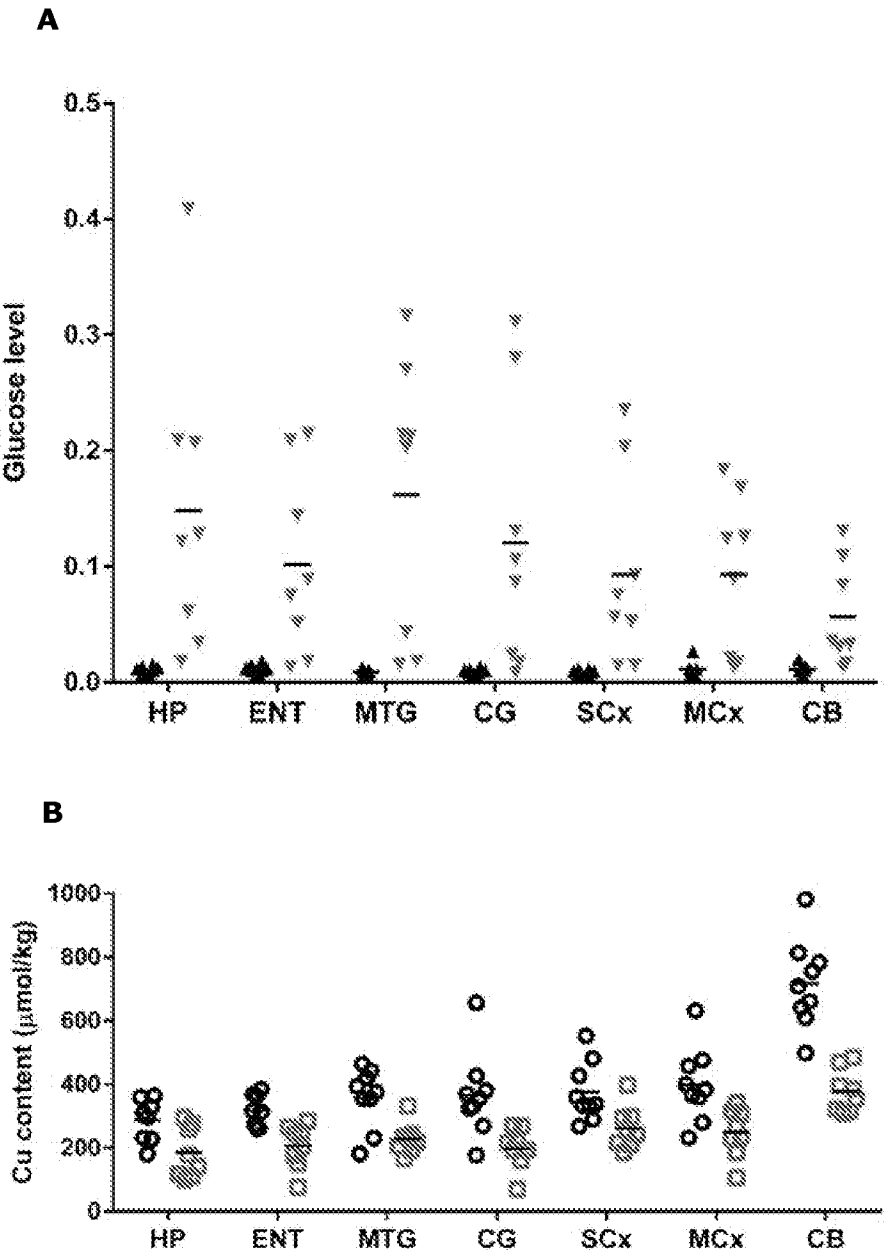
CLAIMS

1. A method of treating Alzheimer's disease (AD) by administration to a mammalian subject in need thereof an effective amount of a copper binding tetramine compound and an effective amount of an agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject.
2. The method of claim 1 wherein the copper binding tetramine compound binds Cu(II).
3. The method of claim 2 wherein the copper binding tetramine compound is a tetramine compound that is specific for Cu(II) over Cu(I).
4. The method of claim 3 wherein the tetramine compound is selected from the group comprising triethylenetetramine (2,2,2 tetramine), 2,3,2 tetramine, 3,3,3 tetramine, and salts, active metabolites, derivatives, and prodrugs thereof.
5. The method of claim 4 wherein the tetramine compound is triethylenetetramine disuccinate.
6. The method of any one of claims 1 to 5 wherein the agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject is selected from the group comprising amylin, an amylin analogue, a GLP-1 agonist, and a selective dipeptidyl peptidase (DPP-IV) inhibitor.
7. The method of claim 6 wherein the amylin analogue is Symmlin.
8. The method of claim 6 wherein the GLP-1 agonist is exenatide, liraglutide, lixisenatide, albiglutide, or dulaglutide, or any combination of two or more thereof.
9. The method of claim 6 wherein the selective dipeptidyl peptidase (DPP-IV) inhibitor is selected from the group comprising Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Anagliptin, Teneligliptin, Alogliptin, Trelagliptin, Gemigliptin, Dutogliptin, and Omarigliptin.
10. The method of claim 6 wherein the selective dipeptidyl peptidase (DPP-IV) inhibitor is selected from the group comprising alogliptin, linagliptin, saxagliptin, sitagliptin, Nesina, Tradjenta, Onglyza, and Januvia.

11. The method of any one of claims 1 to 10 wherein the administration of the copper binding tetramine compound and of the agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in the mammalian subject is simultaneous, sequential, or separate.
12. The method of claim 1 wherein the administration is of triethylenetetramine disuccinate and amylin or Symlin, and may be simultaneous, sequential, or separate administration.
13. The method of claim 1 wherein the administration is of triethylenetetramine disuccinate and one or more agents selected from the group comprising Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Anagliptin, Teneligliptin, Alogliptin, Trelagliptin, Gemigliptin, Dutogliptin, and Omarigliptin, and may be simultaneous, sequential, or separate administration.
14. The method of claim 1 wherein the administration is of triethylenetetramine disuccinate and one or more agents selected from the group comprising alogliptin, linagliptin, saxagliptin, sitagliptin, Nesina, Tradjenta, Onglyza, and Januvia, and may be simultaneous, sequential, or separate administration.
15. The method of any one of claims 1 to 14 wherein the mammalian subject is a non-diabetic subject or a subject who is not undergoing treatment for diabetes.
16. The method of any one of claims 1 to 15 wherein the mammalian subject does not suffer from Wilson's disease.
17. The method of any one of claims 1 to 16 wherein the mammalian subject is a human subject.
18. A pharmaceutical composition comprising one or more copper binding tetramine compounds, additionally comprising or formulated to be administered in conjunction in with an agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic sorbitol, or encephalic fructose in a mammalian subject, wherein the one or more pharmaceutical compositions comprises a pharmaceutically acceptable carrier or diluent.
19. The pharmaceutical composition of claim 18 wherein the tetramine compound is triethylenetetramine disuccinate.
20. The pharmaceutical composition of claim 18 or 19 wherein the agent effective to reduce the amount or concentration of one or more of encephalic glucose, encephalic

- sorbitol, or encephalic fructose in the mammalian subject is selected from the group comprising amylin, an amylin analogue, a GLP-1 agonist, and a selective dipeptidyl peptidase (DPP-IV) inhibitor.
21. The pharmaceutical composition of claim 20 wherein the amylin analogue is Symlin.
 22. The pharmaceutical composition of claim 20 wherein the GLP-1 agonist is exenatide, liraglutide, lixisenatide, albiglutide, or dulaglutide, or any combination of two or more thereof.
 23. The pharmaceutical composition of claim 20 wherein the selective dipeptidyl peptidase (DPP-IV) inhibitor is selected from the group comprising Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Anagliptin, Teneligliptin, Alogliptin, Trelagliptin, Gemigliptin, Dutogliptin, and Omarigliptin.
 24. The pharmaceutical composition of claim 20 wherein the selective dipeptidyl peptidase (DPP-IV) inhibitor is selected from the group comprising alogliptin, linagliptin, saxagliptin, sitagliptin, Nesina, Tradjenta, Onglyza, and Januvia.
 25. The pharmaceutical composition of claim 18 comprising triethylenetetramine disuccinate and amylin or Symlin.
 26. The pharmaceutical composition of claim 18 comprising triethylenetetramine disuccinate and one or more agents selected from the group comprising Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Anagliptin, Teneligliptin, Alogliptin, Trelagliptin, Gemigliptin, Dutogliptin, and Omarigliptin.
 27. The pharmaceutical composition of claim 18 comprising triethylenetetramine disuccinate and one or more agents selected from the group comprising alogliptin, linagliptin, saxagliptin, sitagliptin, Nesina, Tradjenta, Onglyza, and Januvia.

FIGURE 1



INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2017/050013

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/132 (2006.01) A61K 31/00 (2006.01) A61P 25/28 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

TXPDA, TXPEA, TXPEB, TXPEC, TXPEE, TXPEF, TXPEH, TXPEI, TXPEP, TXPEPEA, WPIAP, XPMISC, XPOAC, MEDLINE, EPODOC, HCAPLUS, BIOSIS, EMBASE: tetramine, trientine, Alzheimer, dementia, amyloid, encephalic, brain, cerebral, glucose, sorbitol, fructose, sugar, amylin, symlin, GLP-1, exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, DPP-IV, sitagliptin, vildagliptin, saxagliptin, linagliptin and like terms.

Applicant/Inventor names searched in internal databases provided by IP Australia.

AusPat, Espacenet, PatentScope, PubMed: Cooper Garth

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 7 March 2017		Date of mailing of the international search report 07 March 2017	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer Makiko Umehara AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833142	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/NZ2017/050013
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/115421 A1 (PROTEMIX CORPORATION LIMITED) 02 November 2006 See whole document, in particular abstract, page 12 lines 7-12, page 12 lines 29-30, page 14 lines 12-13, page 16 lines 20-22, page 39 line 14 – page 40 line 6 and page 47 line 28 – page 48 line 12	1-8, 11, 12 and 15-25
Y	See whole document, in particular abstract, page 12 lines 7-12, page 12 lines 29-30, page 14 lines 12-13, page 16 lines 20-22, page 39 line 14 – page 40 line 6 and page 47 line 28 – page 48 line 12	9, 10, 13, 14, 26 and 27
X	WO 2006/104397 A1 (PROTEMIX CORPORATION LIMITED) 05 October 2006 See whole document, in particular abstract, page 10 lines 16-21, page 18 lines 25-26, page 19 lines 23-26, page 25 line 25 – page 26 line 7 and Example 37-48	18-27
X	WO 2006/104401 A1 (PROTEMIX CORPORATION LIMITED) 05 October 2006 See whole document, in particular abstract, page 8 lines 8-13, page 18 lines 5-14, page 28 lines 19-22, page 29 lines 9-24 and Example 37-48	18-27
X	WO 2006/104400 A1 (PROTEMIX CORPORATION LIMITED) 05 October 2006 See whole document, in particular abstract, page 7 lines 18-22, page 13 lines 10-14, page 18 lines 14-28, page 25 lines 3-14 and Examples 1-5	18-24
X	SAITO <i>et al.</i> : "Triethylene-Tetramine (Trien) Therapy for Wilson's Disease" <i>Tohoku Journal of Experimental Medicine</i> , 1991, vol. 164, no. 1, pp. 29-35 See whole document, in particular abstract and page 30 paragraph 3	18-24
Y	LI <i>et al.</i> : "Link Between Type 2 Diabetes and Alzheimer's Disease: From Epidemiology to Mechanism and Treatment" <i>Clinical Interventions in Aging</i> , 2015, vol. 10, pp. 549-560 See whole document, in particular abstract, page 550, left column paragraph 1 and page 556 right column paragraph 2	9, 10, 13, 14, 26 and 27
Y	KORNELIUS <i>et al.</i> : "DPP-4 Inhibitor Linagliptin Attenuates A β -induced Cytotoxicity through Activation of AMPK in Neuronal Cells" <i>CNS Neuroscience & Therapeutics</i> , 2015, vol. 21, no. 7, pp. 549-557 See whole document, in particular abstract and page 556 left column paragraph 2	9, 10, 13, 14, 26 and 27
Form PCT/ISA/210 (fifth sheet) (July 2009)		

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/NZ2017/050013	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2006/115421 A1	02 November 2006	WO 2006115421 A1	02 Nov 2006
		AU 2006240568 A1	02 Nov 2006
		CA 2605930 A1	02 Nov 2006
		EP 1877801 A1	16 Jan 2008
		US 2011136157 A1	09 Jun 2011
WO 2006/104397 A1	05 October 2006	WO 2006104397 A1	05 Oct 2006
WO 2006/104401 A1	05 October 2006	WO 2006104401 A1	05 Oct 2006
WO 2006/104400 A1	05 October 2006	WO 2006104400 A1	05 Oct 2006
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			