The present invention relates to pyrazine derivatives such as those represented by Formulas I and II below.

Formula I

\[ \begin{align*}
X^1 & \quad N \\
Y^1 & \quad X^2
\end{align*} \]

Formula II

\[ \begin{align*}
X^1 & \quad N \\
Y^1 & \quad X^2 \\
Y^3 & \quad X^4
\end{align*} \]

X^1 to X^4 of the compounds of Formulas I and II may be characterized as electron withdrawing groups. In contrast, Y^1 to Y^4 of the compounds of Formulas I and II may be characterized as electron donating groups. Pyrazine derivatives of the present invention may be utilized in assessing renal function. In particular, an effective amount of a pyrazine derivative of the invention may be administered into a body of a patient. The pyrazine derivative that is in the body may be exposed to visible and/or infrared light to cause spectral energy to emanate from the pyrazine derivative. This emanating spectral energy may be detected and utilized to determine renal function of the patient.
Creatinine (1)
MW: 113

o-Iodohippurate (2)
MW: 327

$^{99m}$Tc-DTPA (3)
MW: 487

$^{99m}$Tc-MAG3 (4)
MW: 364

Fig. 1
PYRAZINE DERIVATIVES AND USES THEREOF IN RENAL MONITORING

REFERENCE TO RELATED APPLICATIONS


FIELD OF THE INVENTION

The present invention relates to pyrazine derivatives capable of absorbing and emanating spectral energy in the visible and/or near infrared spectrum. In addition, the present invention relates to methods of using non-radioactive, exogenous agents such as the previously mentioned pyrazine derivatives in medical procedures (e.g., the monitoring of renal function).

BACKGROUND

As a preliminary note, various publications are referenced throughout this disclosure by Arabic numerals in brackets. A citation corresponding to each reference number is listed following the detailed description.

Acute renal failure (ARF) is a common ailment in patients admitted to general medical-surgical hospitals. Approximately half of the patients who develop ARF die, and survivors face marked increases in morbidity and prolonged hospitalization [1]. Early diagnosis is generally believed to be important, because renal failure is often asymptomatic and typically requires careful tracking of renal function markers in the blood. Dynamic monitoring of renal functions of patients is desirable in order to minimize the risk of acute renal failure brought about by various clinical, physiological and pathological conditions [2-6]. Such dynamic monitoring tends to be particularly important in the case of critically ill or injured patients, because a large percentage of these patients tend to face risk of multiple organ failure (MOF) potentially resulting in death [7, 8]. MOF is a sequential failure of the lungs, liver and kidneys and is incited by one or more of acute lung injury (ALI), adult respiratory distress syndrome (ARDS), hypermetabolism, hypotension, persistent inflammatory focus and sepsis syndrome. The common histological features of hypotension and shock leading to MOF generally include tissue necrosis, vascular congestion, interstitial and cellular edema, hemorrhage and microthrombi. These changes generally affect the lungs, liver, kidneys, intestine, adrenal glands, brain and pancreas in descending order of frequency [9]. The transition from early stages of trauma to clinical MOF generally corresponds with a particular degree of liver and renal failure as well as a change in mortality risk from about 30% up to about 50% [10].

Traditionally, renal function of a patient has been determined using crude measurements of the patient's urine output and plasma creatinine levels [11-13]. These values are frequently misleading because such values are affected by age, state of hydration, renal perfusion, muscle mass, dietary intake, and many other clinical and anthropometric variables. In addition, a single value obtained several hours after sampling may be difficult to correlate with other physiologic events such as blood pressure, cardiac output, state of hydration and other specific clinical events (e.g., hemorrhage, bacteremia, ventilator settings and others).

With regard to conventional renal monitoring procedures, an approximation of a patient's glomerular filtration rate (GFR) can be made via a 24 hour urine collection procedure that (as the name suggests) typically requires about 24 hours for urine collection, several more hours for analysis, and a meticulous bedside collection technique. Unfortunately, the undesirably late timing and significant duration of this conventional procedure can reduce the likelihood of effectively treating the patient and/or saving the kidney(s). As a further drawback to this type of procedure, repeat data tends to be equally as cumbersome to obtain as the originally acquired data.

Occasionally, changes in serum creatinine of a patient must be adjusted based on measurement values such as the patient's urinary electrolytes and osmolality as well as derived calculations such as "renal failure index" and/or "fractional excretion of sodium." Such adjustments of serum creatinine undesirably tend to require contemporaneous collection of additional samples of serum and urine and, after some delay, further calculations. Frequently, dosing of medication is adjusted for renal function and thus can be equally as inaccurate, equally delayed, and as difficult to reassess as the measurement values and calculations upon which the dosing is based. Finally, clinical decisions in the critically ill population are often equally as important in their timing as they are in their accuracy.

It is known that hydrophilic, anionic substances are generally capable of being excreted by the kidneys [14]. Renal clearance typically occurs via two pathways: glomerular filtration and tubular secretion. Tubular secretion may be characterized as an active transport process, and hence, the substances clearing via this pathway typically exhibit specific properties with respect to size, charge and lipophilicity.

Most of the substances that pass through the kidneys are filtered through the glomerulus (a small intertwined group of capillaries in the malpighian body of the kidney). Examples of exogenous substances capable of clearing the kidney via glomerular filtration (hereinafter referred to as "GFR agents") are shown in FIG. 1 and include creatinine (1), o-iodohippuran (2), and 99mTc-DTPA (3) [15-17]. Examples of exogenous substances that are capable of undergoing renal clearance via tubular secretion include 99mTc-MAG3 (4) and other substances known in the art [15, 18, 19]. 99mTc-MAG3 (4) is also widely used to assess renal function though gamma scintigraphy as well as through renal blood flow measurement. As one drawback to the substances illustrated in FIG. 1, o-iodohippuran (2), 99mTc-DTPA (3) and 99mTc-MAG3 (4) include radioisotopes to enable the same to be detected. Even
if non-radioactive analogs (e.g., such as an analog of o-iodohippurin (2)) or other non-radioactive substances were to be used for renal function monitoring, such monitoring would typically require the use of undesirable ultraviolet radiation for excitation of those substances.

SUMMARY

In one regard, the present invention relates to transforming lipophilic fluorescent dyes into hydrophilic molecules. One concept of the present invention relates to molecules whose clearance properties are preferably similar to that of creatinine or o-iodohippurin, and to render such molecules hydrophilic by incorporating appropriate polar functionalities such as hydroxyl, carboxyl, sulfonate, phosphonate and the like into their backbones. Pyrazine dyes of the invention may be characterized by some as being desirable for renal applications because they tend to be cleared from the body via the kidneys, demonstrate absorption and emission/fluorescence in the visible region, and tend to exhibit significant Stokes shifts. These properties allow flexibility in both tuning a molecule to a desired wavelength and introducing a variety of substituents to improve clearance properties.

![Formula I]

![Formula A]

In a first aspect, the present invention is directed to pyrazine derivatives of Formula I. With regard to Formula I, X' and X" may be characterized as electron withdrawing substituents and may be independently chosen from the group consisting of —CN, —CO₂R', —CONR²R³, —COR, —NO₂, —SOR³, —SO₂R³, —SO₃R², —PO₃R²R³, —CONH(AA), and —CONH(PS). In some embodiments, at least one of X' and X" is either —CONH(AA) or —CONH(PS). (AA) is a polypeptide chain that includes one or more natural or unnatural α-amino acids linked together by peptide bonds. (PS) is a sulfated or non-sulfated polysaccharide chain that includes one or more monosaccharide units connected by glycosidic linkages. Y³ and Y⁴ may, at least in some embodiments, be characterized as electron donating substituents and may be independently chosen from the group consisting of —OR¹⁰, —SR¹¹, —NR²R¹³, —N(R¹⁴COR¹⁵, —P(R¹⁶)³, —P(OR¹⁷)³, and substituents corresponding to Formula A above. In some embodiments, at least one of Y¹ and Y² is either —P(R¹⁸)³ or —P(R¹⁷)³. Z¹ may be a single bond, —CR²R¹⁹, —O, —NR²₀, —NCR²¹, —S, —SO and —SO₂. R¹ to R² may independently be any one of a hydrogen atom, an anionic functional group (e.g., carboxylate, sulfonate, sulfate, phosphonate and phosphate) or a hydrophilic functional group (e.g., hydroxyl, carboxyl, sulfonate, sulfonato and phosphonato).

A second aspect of the invention is directed to pyrazine derivatives of Formula II. With regard to Formula II, X¹ and X⁴ may be characterized as electron withdrawing substituents and may be independently chosen from the group consisting of —CN, —CO₂R²², —CONR²³R²⁴, —COR²⁵, —NO₂, —SOR²⁶, —SO₂R²⁷, —SO₃R²⁸, —PO₃R²R²³, —CONH(AA), and —CONH(PS). In some embodiments, at least one of X¹ and X⁴ is either —CONH(AA) or —CONH(PS). (AA) is a polypeptide chain that includes one or more natural or unnatural α-amino acids linked together by peptide bonds. (PS) is a sulfated or non-sulfated polysaccharide chain that includes one or more monosaccharide units connected by glycosidic linkages. Y¹ and Y⁴ may, at least in some embodiments, be characterized as electron donating substituents and may be independently chosen from the group consisting of —OR³¹, —SR³², —NR³³R³⁴, —N(R³⁵COR³⁶, —P(R³⁷)³, —P(OR³⁸)³, and substituents corresponding to Formula B above. In some embodiments, at least one of Y¹ and Y⁴ is either —P(R³⁷)³ or —P(OR³⁸)³. Z² may be a single bond, —CR³⁹R⁴⁰, —O, —NR³¹, —NCR³², —S, —SO and —SO₂. R² to R³² may be any suitable substituents capable of providing and/or enhancing desired biological and/or physicochemical properties of pyrazine derivatives of Formula I. For instance, for renal function assessment, each of the R groups of R¹ to R²¹ may independently be any one of a hydrogen atom, an anionic functional group (e.g., carboxylate, sulfonate, sulfate, phosphonate and phosphate) or a hydrophilic functional group (e.g., hydroxyl, carboxyl, sulfonate, sulfonato and phosphonato). As an example, in some embodiments, R¹ to R³² may independently be selected from
the group consisting of —H, —(CH₂)₂OR⁵¹, —CH₃(CHOH)₉R⁵², —CH₂(CH(OH)₉COH, —(CH₂O)₂H, CO₂H, —(CH₃)₂NR⁺R⁵⁴, —CH₂(NH₂)H₂, —CH₂NR⁺R⁵⁶, —(CH₂CH₂O)₂R⁵⁷, —(CH₂)₂CO(CH₂CH₂O)₂R⁵⁸, —(CH₂)₄SO₄H, —(CH₂)₄SO₄⁻, —(CH₂)₄OSO₂H, —(CH₂)₄OSO₂⁻, —(CH₂)₄NHSO₂H, —(CH₂)₄NHSO₂⁻, —(CH₂)₄PO₄H₂, —(CH₂)₄PO₄⁻, —(CH₂)₄PO₅H, —(CH₂)₄PO₅⁻, —(CH₂)₄PO₆H₂, —(CH₂)₄PO₆⁻ and —(CH₂)₄PO₆²⁻. In such embodiments, each of R⁵¹ to R⁵₈ may independently be —H or —CH₃, ‘e’, ‘g’, ‘h’, ‘p’ and ‘q’ may be any appropriate integers. For instance, in some embodiments, ‘e’, ‘f’, and ‘h’ may independently vary from 1 to 10, ‘g’ may vary from 1 to 100, and ‘p’ and ‘q’ may independently vary from 1 to 3.

Yet a third aspect of the invention is directed to pharmaceutically acceptable compositions, each of which includes one or more pyrazine derivatives disclosed herein. Incidentally, the phrase “pharmaceutically acceptable” herein refers to substances which are, within the scope of sound medical judgment, suitable for use in contact with relevant tissues of humans and animals without undue toxicity, irritation, allergic response, and are commensurate with a reasonable benefit/risk ratio. The compositions of this third aspect may include one or more appropriate excipients such as, but not limited to, suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. One example of a composition of this third aspect may include at least one pyrazine derivative of Formula I and at least one pyrazine derivative of Formula II. Another example of a composition of the third aspect may include one or more pyrazine derivatives of Formula I or one or more pyrazine derivatives of Formula II.

Still a fourth aspect of the invention is directed to methods of determining renal function using pyrazine derivatives such as those described above with regard to Formulas I and II. In these methods, an effective amount of a pyrazine derivative is administered into the body of a patient (e.g., a mammal such as a human or animal subject). Incidentally, an “effective amount” herein generally refers to an amount of pyrazine derivative that is sufficient to enable renal clearance to be analyzed. The pyrazine derivative in the body of the patient is exposed to at least one of visible and near infrared light. Due to this exposure of the pyrazine derivative to the visible and/or infrared light, the pyrazine derivative emanates spectral energy that may be detected by appropriate detection equipment. This spectral energy emanating from the pyrazine derivative may be detected using an appropriate detection mechanism such as an invasive or non-invasive optical probe. Herein, “emanating” or the like refers to spectral energy that is emitted and/or fluoresced from a pyrazine derivative. Renal function can be determined based on the spectral energy that is detected. For example, an initial amount of the pyrazine derivative present in the body of a patient may be determined by a magnitude/intensity of light emanated from the pyrazine derivative that is detected (e.g., in the bloodstream). As the pyrazine derivative is cleared from the body, the magnitude/intensity of detected light generally diminishes. Accordingly, a rate at which this magnitude of detected light diminishes may be correlated to a renal clearance rate of the patient. This detection may be done periodically or in substantially real time (providing a substantially continuous monitoring of renal function). Indeed, methods of the present invention enable renal function/clearance to be determined via detecting one or both a change and a rate of change of the detected magnitude or spectral energy (indicative of an amount of the pyrazine derivative that has not been cleared) from the portion of the pyrazine derivative that remains in the body. While this fourth aspect has been described with regard to use of a single pyrazine derivative of the invention, it should be noted that some embodiments of this fourth aspect include the use of compositions of the invention that may include one or more pyrazine derivatives disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 illustrates some structures of conventional renal agents.

FIG. 2 illustrates a block diagram of an assembly for assessing renal function.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

As mentioned above, the present invention includes pyrazine derivatives of Formula I. In a first family of embodiments, X₁ and X₂ are independently selected from the group consisting of —CN, —CO₂R, —CONR₂, —COR¹, —NO₂, —SOR, —SO₂R¹, —SO₂OR, —PO₃R²R⁶, —CONH(AA), and —CONH(PS), wherein at least one of (e.g., one or both of) X₁ and X₂ is independently either —CONH(AA) or —CONH(PS). For instance, in one group of embodiments, at least one of X₁ and X₂ is —CONH(PS). With further regard to this first family of embodiments, Y¹ and Y² are independently selected from the group consisting of —OR¹¹, —SR¹¹, —NR¹²R¹³, —N(R¹⁴)COR¹⁵, —P(R¹⁶)₃, —P(OR¹⁷)₃, and substituents corresponding to Formula A above.

In some embodiments of this first family, X₁ and X₂ are independently selected from the group consisting of —CN, —CO₂R, —CONR₂, —CONH(AA), and —CONH(PS), wherein at least one of X₁ and X₂ is independently either —CONH(AA) or —CONH(PS). Y¹ and Y₂ of some embodiments of the first family are independently selected from the group consisting of —NR¹²R¹³ and substituents corresponding to Formula A above.

In a second family of embodiments, X₁ and X₂ are independently selected from the group consisting of —CN, —CO₂R, —CONR₂, —COR¹, —NO₂, —SOR, —SO₂R¹, —SO₂OR, —PO₃R²R⁶, —CONH(AA), and
—CONH(PS). Further, Y₁ and Y₂ are independently selected from the group consisting of —OR₁⁰, —SR₁¹, —NR₁²R₁³, —NR₁⁴COR₁⁵, —P(R₁⁶)₃, —P(OR₁⁷)₃, P(OR₁⁷)₃, and substituents corresponding to Formula A above, wherein at least one of (e.g., one of or both of) Y₁ and Y₂ is independently either —P(R₁⁶)₃ or —P(OR₁⁷)₃. In other words, in one group of embodiments, at least one of Y₁ or Y₂ is —P(R₁⁶)₃. In another group of embodiments, at least one of Y₁ and Y₂ is —P(OR₁⁷)₃.

In some embodiments of this second family, X¹ and X² are independently selected from the group consisting of —CN, —CO₂R, —COR, —CONH(AA), and —CONH(PS). In some embodiments of the second family, one of Y₁ and Y₂ is —P(R₁⁶)₃ or —P(OR₁⁷)₃, and the other of Y₁ and Y₂ is —NR₁²R₁³ or a substituent corresponding to Formula A above.

With regard to the above-described first and second families, Z¹ is selected from the group consisting of a single bond, —CR₁⁸R₁⁹, —O, —NR₂⁰, —NCOR₂¹, —S, —SO and —SO₂. In some embodiments, Z¹ is selected from the group consisting of —O, —NR₂⁰, —S, —SO and —SO₂. In other embodiments, Z¹ is selected from the group consisting of —O and —NR₂⁰.

R¹ to R²¹ of the first and second families are independently selected from the group consisting of —H, —(CH₂)₃OR, —(CH₂)₄(OH)CO₂H, —(CH₂)₅CO₂H, —(CH₂)₆CO₂H, —(CH₂)₇CO₂H, —(CH₂)₈CO₂H, —(CH₂)₉CO₂H, —(CH₂)₁₀CO₂H, —(CH₂)₁₁CO₂H, —(CH₂)₁₂CO₂H, —(CH₂)₁₃CO₂H, —(CH₂)₁₄CO₂H, —(CH₂)₁₅CO₂H, —(CH₂)₁₆CO₂H, —(CH₂)₁₇CO₂H, —(CH₂)₁₈CO₂H, —(CH₂)₁₉CO₂H, —(CH₂)₂₀CO₂H, —(CH₂)₂₁CO₂H, —(CH₂)₂²CO₂H, —(CH₂)₂₃CO₂H, —(CH₂)₂₄CO₂H, —(CH₂)₂₅CO₂H, —(CH₂)₂₆CO₂H, —(CH₂)₂₇CO₂H, —(CH₂)₂₈CO₂H, —(CH₂)₂₉CO₂H, —(CH₂)₃₀CO₂H, —(CH₂)₃₁CO₂H, —(CH₂)₃₂CO₂H, —(CH₂)₃₃CO₂H, —(CH₂)₃₄CO₂H, —(CH₂)₃₅CO₂H, —(CH₂)₃₆CO₂H, —(CH₂)₃₇CO₂H, —(CH₂)₃₈CO₂H, —(CH₂)₃₉CO₂H, —(CH₂)₄₀CO₂H, —(CH₂)₄¹CO₂H, —(CH₂)₄²CO₂H, —(CH₂)₄₃CO₂H, —(CH₂)₄₄CO₂H, —(CH₂)₄₅CO₂H, —(CH₂)₄₆CO₂H, —(CH₂)₄₇CO₂H, —(CH₂)₄₈CO₂H, —(CH₂)₄₉CO₂H, —(CH₂)₅₀CO₂H, —(CH₂)₅¹CO₂H, —(CH₂)₅²CO₂H, —(CH₂)₅₃CO₂H, —(CH₂)₅₄CO₂H, —(CH₂)₅₅CO₂H, —(CH₂)₅₆CO₂H, —(CH₂)₅₇CO₂H, —(CH₂)₅₈CO₂H, —(CH₂)₅₉CO₂H, —(CH₂)₆₀CO₂H, —(CH₂)₆¹CO₂H, —(CH₂)₆₂CO₂H, —(CH₂)₆₃CO₂H, —(CH₂)₆₄CO₂H, —(CH₂)₆₅CO₂H, —(CH₂)₆₆CO₂H, —(CH₂)₆₇CO₂H, —(CH₂)₆₈CO₂H, —(CH₂)₆₉CO₂H, —(CH₂)₇₀CO₂H, —(CH₂)₇¹CO₂H, —(CH₂)₇₂CO₂H, —(CH₂)₇₃CO₂H, —(CH₂)₇₄CO₂H, —(CH₂)₇₅CO₂H, —(CH₂)₇₆CO₂H, —(CH₂)₇₇CO₂H, —(CH₂)₇₈CO₂H, —(CH₂)₇₉CO₂H, —(CH₂)₈₀CO₂H, —(CH₂)₈₁CO₂H, —(CH₂)₈₂CO₂H, —(CH₂)₈₃CO₂H, —(CH₂)₈₄CO₂H, —(CH₂)₈₅CO₂H, —(CH₂)₈₆CO₂H, —(CH₂)₈₇CO₂H, —(CH₂)₈₈CO₂H, —(CH₂)₈₉CO₂H, —(CH₂)₉₀CO₂H, —(CH₂)₉₁CO₂H, —(CH₂)₉₂CO₂H, —(CH₂)₉₃CO₂H, —(CH₂)₉₄CO₂H, —(CH₂)₉₅CO₂H, —(CH₂)₉₆CO₂H, —(CH₂)₉₇CO₂H, —(CH₂)₉₈CO₂H, —(CH₂)₉₉CO₂H, —(CH₂)₁₀₀CO₂H, and substituents corresponding to Formula A above.

The present invention is also includes pyrimidine derivatives corresponding to Formula II above. In a third family of embodiments of the invention, X² and X³ are independently selected from the group consisting of —CN, —CO₂R, —CONR₂², —COR₂³, —NO₂, —SOR₂⁶, —SO₃R₂⁷, —SO₂OR₂₉, —PO₂R₂⁴R₂₈, —PO₃R₂⁷R₃₀, —CONH(AA), and —CONH(PS), wherein at least one of (e.g., one of or both of) X² and X³ is independently selected from the group consisting of —OR₂⁹, —SR₂⁵, —NR₂⁷R₂₈, —N(R₂⁵)COR₂⁶, —P(R²⁷)₃, —P(OR₂⁸)₃, and substituents corresponding to Formula B above.
In some embodiments of this third family, X^3 and X^4 are independently selected from the group consisting of —CN, —COOR^22, —CONR^23R^24, —CONH(AA), and —CONH(PS), wherein at least one of X^3 and X^4 is independently either —CONH(AA) or —CONH(PS). Y^3 and Y^4 of some embodiments of the third family are independently selected from the group consisting of —NR^31R^32 and substituents corresponding to Formula B above.

In still a fourth family of embodiments, X^3 and X^4 are independently selected from the group consisting of —CN, —COOR^22, —CONR^23R^24, —CONH(AA), and —CONH(PS). Y^3 and Y^4 are independently selected from the group consisting of —OR^31, —SR^32, —NR^31R^32, —N[R^35]COR^36, —P(R^37)_3, —P(OR^38)_3, and substituents corresponding to Formula B above, wherein at least one of Y^3 and Y^4 is independently either —P(R^37)_3 or —P(OR^38)_3. For instance, in one group of embodiments, at least one of Y^3 and Y^4 is —P(R^37)_3. In another group of embodiments, at least one of Y^3 and Y^4 is —P(OR^38)_3.

In some embodiments of this fourth family, X^3 and X^4 are independently selected from the group consisting of —CN, —COOR^22, —CONR^23R^24, —CONH(AA), and —CONH(PS). In some embodiments of the fourth family, one of Y^3 and Y^4 is —P(R^37)_3 or —P(OR^38)_3, and the other of Y^3 and Y^4 is —NR^31R^32 or a substituent corresponding to Formula B above.

With regard to the above-described third and fourth families, Z^2 is selected from the group consisting of a single bond, —CR^39—R^40, —OR^41, —NR^42—, —S—, —SO—, and —SO_2. In some embodiments, Z^2 is selected from the group consisting of —O—, —NR^41—, —S—, —SO—, and —SO_2. In some embodiments, Z^2 is selected from the group consisting of —O— and —NR^41—.

R^22 to R^24 are independently selected from the group consisting of —H, —(CH_2)_2OR^25, —CH_2(CH(OH))R^25, —CH_2(CH(OH))_2COH, —(CH_2)_2NR^26—R^26, —CH(CH_2)_2NH_2COH, —CH(CH_2)_2NHCH_2OH, —CH(CH_2)_2NR^26—R^26, —(CH_2)_2CH(OH)R^27, —(CH_2)_2CO(CH_2CH_2O)_2R^28, —(CH_2)_2SO_2H, —(CH_2)_2SO_2—, —(CH_2)_2OSO_2H, —(CH_2)_2OSO_2—, —(CH_2)_2NHSO_3H, —(CH_2)_2NHSO_3—, —(CH_2)_2PO(OH)_2, —(CH_2)_2PO(OH)—, —(CH_2)_2PO(O)H_2, —(CH_2)_2PO(O)H—, and —(CH_2)_2PO(O)PO_3H. In such embodiments, R^25 to R^28 are independently —H or —CH_3. In one group of embodiments, R^22 to R^24 are independently selected from the group consisting of —H, —(CH_2)_2OR^25, —CH_2(CH(OH))_2COH, —(CH_2)_2NR^26—R^26, —CH(CH_2)_2NH_2COH, —CH(CH_2)_2NHCH_2OH, —CH(CH_2)_2NR^26—R^26, —(CH_2)_2CH(OH)R^27, —(CH_2)_2CO(CH_2CH_2O)_2R^28, and —(CH_2)_2SO_2H. In another group of embodiments, R^22 to R^24 are independently selected from the group consisting of —H, —(CH_2)_2OR^25, —CH_2(CH(OH))_2COH, —(CH_2)_2NR^26—R^26, —(CH_2)_2CO(CH_2CH_2O)_2R^28, and —(CH_2)_2SO_2H. In still another group of embodiments, R^22 to R^24 are independently selected from the group consisting of —H, —(CH_2)_2OR^25, —CH_2(CH(OH))_2COH, —(CH_2)_2NR^26—R^26, and —(CH_2)_2CO(CH_2CH_2O)_2R^28.

Still with regard to the third and fourth families, ‘e’, ‘f’, ‘g’, and ‘h’ independently vary from 1 to 10, ‘g’ varies from 1 to 100, and ‘p’ and ‘q’ independently vary from 1 to 3. In some embodiments, ‘e’, ‘f’, ‘g’, and ‘h’ independently vary from 1 to 6. In some embodiments, ‘g’ varies from 1 to 20. In some embodiments, ‘m’ and ‘n’ are independently 0 or 1.

As with the first and second families of embodiments described above, (AA) of the third and fourth families is a polypeptide chain including one or more natural or unnatural α-amino acids linked by peptide bonds. Accordingly, the description of (AA) with reference to the first and second families of embodiments applies to (AA) of the third and fourth families of embodiments as well. Likewise, (PS) of the third and fourth families is a sulfated or nonsulfated polysaccharide chain including one or more monosaccharide units connected by glycosidic linkages. As such, the description of (PS) with reference to the first and second families of embodiments above applies to (PS) of the third and fourth families of embodiments as well.

Syntheses of pyrazine derivatives, in general, have been previously studied [27] and described [25, 26, 28, 29]. Preparation procedures for some of the pyrazine derivatives of the present invention, using procedures similar to the above references, are described later in Examples 1 to 11. It is noteworthy that the alkylation of the electron donating amino group in cyano- or carboxy-pyrazines has a profound effect on electronic transition of the pyrazine chromophore in that the diazalkylation of the amino group in 2,5-diamino-3,5-dicyano-pyrazine produces large bathochromic shift of about 40-60 nm. It is also noteworthy that the pyridoline and piperdikido derivatives exhibit substantial difference in their UV spectra in that the former exhibits a bathochromic shift of about 34 nm. These results could be explained on the basis that the highest occupied molecular orbital (HOMO) of the alkylated aminopyrazine is destabilized compared to the parent amino compound. Therefore, based on the above premise, it is predicted that pyrazine derivatives containing highly strained azacycloalkyl substituents, which were not disclosed previously, exhibit larger bathochromatic shifts compared to unstrained cyclic analogs.

In accordance with the present invention, one protocol for assessing physiological function of body cells includes administering an effective amount of a pyrazine derivative represented by Formula I or II into a body of a patient. An appropriate dosage of the pyrazine that is administered to the patient is readily determinable by one of ordinary skill in the art and may vary according to the clinical procedure contemplated, generally ranging from about 1 nanomolar to about 100 micromolar. The administration of the pyrazine derivative to the patient may occur in any of a number of appropriate fashions including, but not limited to: (1) intravenous, intraperitoneal, or subcutaneous injection or infusion; (2) oral administration; (3) transdermal absorption through the skin; and (4) inhalation.

Pyrazine derivatives of this invention can be administered as solutions in most pharmaceutically acceptable intravenous vehicles known in the art. Pharmaceutically acceptable vehicles that are well known to those skilled in the art include, but are not limited to, 0.01-0.1 M phosphate buffer or 0.8% saline. Additionally, pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, emulsions, or appropriate combinations thereof. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic.
esters such as ethyl oleate. Examples of aqueous carriers are water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Exemplary parenteral vehicles include sodium chloride solution, Ringer’s dextrose, dextrose and sodium chloride, lactated Ringer’s or fixed oils. Exemplary intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer’s dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, collating agents, inert gases and the like.

Suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers are also suitable excipients. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronics F68, bile acid salts), solubilizing agents (e.g., glycerol, polyethylene glycol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or toxicity modifiers (e.g., lactose, mannitol), complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polyactic acid, polyglycolic acid, hydrogels, etc., or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spherooplasts. Such compositions may likely influence the physical state, solubility, stability, rate of in vivo release, and/or rate of in vivo clearance.

Still referring to the above-mentioned protocol, the pyrazine derivative is exposed to visible and/or near infrared light. This exposure of the pyrazine derivative to light may occur at any appropriate time but preferably occurs while the pyrazine derivative is located in the body. Due to this exposure of the pyrazine derivative to the visible and/or infrared light, the pyrazine derivative emanates spectral energy (e.g., visible and/or near infrared light) that may be detected by appropriate detection equipment. The spectral energy emanated from the pyrazine derivative tends to exhibit a wavelength range greater than a wavelength range absorbed by the pyrazine derivative. For example, if an embodiment of the pyrazine derivative absorbs light of about 700 nm, the pyrazine derivative may emit light of about 745 nm.

Detection of the pyrazine derivative (or more particularly, the light emanating therefrom) may be achieved through optical fluorescence, absorbance or light scattering procedures known in the art. In one embodiment, this detection of the emanated spectral energy may be characterized as a collection of the emanated spectral energy and a generation of electrical signal indicative of the collected spectral energy. The mechanism(s) utilized to detect the spectral energy from the pyrazine derivative that is present in the body may be designed to detect only selected wavelengths (or wavelength ranges) and/or may include one or more appropriate spectral filters. Various catheters, endoscopes, ear clips, hand bands, head bands, surface coils, finger probes and the like may be utilized to expose the pyrazine derivative to light and/or to detect the light emanating therefrom [30]. This detection of spectral energy may be accomplished at one or more times intermittently or may be substantially continuous.

Renal function of the patient can be determined based on the detected spectral energy. This can be achieved by using data indicative of the detected spectral energy and generating an intensity/time profile indicative of a clearance of the pyrazine derivative from the body. This profile may be correlated to a physiological or pathological condition. For example, the patient’s clearance profiles and/or clearance rates may be compared to known clearance profiles and/or rates to assess the patient’s renal function and to diagnose the patient’s physiological condition. In the case of analyzing the presence of the pyrazine derivative in bodily fluids, concentration/time curves may be generated and analyzed (preferably in real time) using an appropriate microprocessor to diagnose renal function.

Physiological function can be assessed by: (1) comparing differences in manners in which normal and impaired cells remove a pyrazine derivative of the invention from the bloodstream; (2) measuring a rate or an accumulation of a pyrazine derivative of the invention in the organs or tissues; and/or (3) obtaining tomographic images of organs or tissues having a pyrazine derivative of the invention associated therewith. For example, blood pool clearance may be measured non-invasively from convenient surface capillaries such as those found in an earlobe or a finger or can be measured invasively using an appropriate instrument such as an endovascular catheter.

Accumulation of a pyrazine derivative of the invention within cells of interest can be assessed in a similar fashion.

A modified pulmonary artery catheter may also be utilized to, inter alia, make the desired measurements [32] of spectral energy emanating from a pyrazine derivative of the invention. The ability for a pulmonary catheter to detect spectral energy emanating from a pyrazine derivative of the invention is a distinct improvement over current pulmonary artery catheters that measure only intravascular pressures, cardiac output and other derived measures of blood flow. Traditionally, critically ill patients have been managed using only the above-listed parameters, and their treatment has tended to be dependent upon intermittent blood sampling and testing for assessment of renal function. These traditional parameters provide for discontinuous data and are frequently misleading in many patient populations.

Modification of a standard pulmonary artery catheter only requires making a fiber optic sensor thereof of wavelength-specific. Catheters that incorporate fiber optic technology for measuring mixed venous oxygen saturation exist currently. In one characterization, it may be said that the modified pulmonary artery catheter incorporates a wavelength-specific optical sensor into a tip of a standard pulmonary artery catheter. This wavelength-specific optical sensor can be utilized to monitor renal function-specific elimination of a designed optically detectable chemical entity such as the pyrazine derivatives of the present invention. Thus, by a method analogous to a dye dilution curve, real-time renal function can be monitored by the disappearance/clearance of an optically detected compound.

The following examples illustrate specific embodiments of the invention. As would be apparent to skilled artisans, vari-
ous changes and modifications are possible and are contemplated within the scope of the invention described.

Example 1

Preparation of 3,6-diamino-N$_2$N$_3$N$_4$N$_5$-tetrakis(2-methoxyethyl)pyrazine-2,5-dicarboxamide

A mixture of 3,6-diaminopyrazine-2,5-dicarboxylic acid (200 mg, 1.01 mmol), bis-2-(methoxyethyl)amine (372 µL, 335.5 mg, 2.52 mmol), HOBr-H$_2$O (459 mg, 3.00 mmol), and EDC-HCl (575 mg, 3.00 mmol) were stirred together in DMF (20 mL) for 1 h at room temperature. The mixture was concentrated to dryness and the residue was partitioned with EtOAc and water. The layers were separated and the EtOAc solution was washed with saturated NaHCO$_3$ and brine. The solution was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated. Purification by radial flash chromatography (SiO$_2$, 10/1 CHCl$_3$-MeOH) afforded 228.7 mg (53% yield) of Example 1 as an orange foam: $^1$H NMR (300 MHz, CDCl$_3$) 0.49 (s, 4H), 3.37 (apparent t, J=5.4 Hz, 4H), 3.70 (apparent t, J=5.4 Hz, 4H), 3.56 (apparent t, J=5.4 Hz, 3.67 (s, 6H), 3.28 (s, 6H), 13$^3$C NMR (75 MHz, CDCl$_3$) 0.16 (s), 1.10 (s), 1.90 (s), 70.8 (t), 59.2 (q), 49.7 (t), 47.1 (t). LCMS (5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=3.14 min on 30 nm column, (M+H)$^+$=429. UV/vis (100 µM in PBS) $\lambda_{max}$=394 nm. Fluorescence (100 nm) $\lambda_{max}$=394 nm $\lambda_{em}$=550 nm.

Example 2

3,6-diamino-N$_2$N$_3$N$_4$-bis(2,3-dihydroxypropyl)pyrazine-2,5-dicarboxamide

A mixture of 3,6-diaminopyrazine-2,5-dicarboxylic acid (350 mg, 1.77 mmol), racemic (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (933 µL, 944 mg, 7.20 mmol), HOBr-H$_2$O (812 mg, 5.33 mmol), and EDC-HCl (1.02 g, 5.32 mmol) were stirred together in DMF (20 mL) for 16 h at room temperature. The mixture was concentrated to dryness and the residue was partitioned with EtOAc and water. The layers were separated and the EtOAc solution was washed with saturated NaHCO$_3$ and brine. The solution was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to afford 665 mg (88% yield) of the bis-amide diastereomeric pair as a yellow solid: $^1$NMR (300 MHz, CDCl$_3$) 0.83 (t, J=5.8 Hz, 2H), 6.55 (s, 4H), 4.21 (quintet, J=5.8 Hz, 2H), 3.98 (dd, J=8.4 Hz, 6.3 Hz, 2H), 3.65 (dd, J=8.4 Hz, J=5.8 Hz, 2H), 3.39 (apparent quartet-diastereotopic mixture, J=5.9 Hz, 4H), 1.35 (s, 6H), 1.26 (s, 6H), 13$^3$C NMR (75 MHz, CDCl$_3$) 0.16 (s), 146.8 (s), 126.8 (s), 109.2 (s), 74.8 (d), 67.2 (t), 42.2, 41.1 (t-diastereotopic pair), 27.6 (q), 26.1 (q).

Step 2

The product from Step 1 was dissolved in THF (100 mL) and treated with 1.0 N HCl (2 mL). After hydrolysis was complete, the mixture was treated with K$_2$CO$_3$ (1 g) and stirred for 1 h and filtered through a plug of C18 with using methanol. The filtrate was concentrated to dryness and the residue was triturated with MeOH (50 mL). The solids were filtered and discarded and the residue was treated with ether (50 mL). The precipitate was collected by filtration and dried at high vacuum. This material was purified by radial flash chromatography to afford 221 mg (36% yield) of Example 2 as an orange solid: $^1$NMR (300 MHz, DMSO-d$_6$), 0.80 (bm, 6H), 5.39 (bs, 2H), 4.88 (bs, 2H), 3.63-3.71 (complex, 2H), 3.40 (dd, J=11.1, 5.10 Hz, 2H), 3.28 (dd, J=11.1, 6.60 Hz, 2H), 2.92 (dd, J=12.6, 3.3 Hz, 2H), 2.65 (dd, J=12.6, 8.4 Hz, 2H), LCMS (5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=4.13 min on 30 nm
A mixture of sodium 3,6-diaminopyrazine-2,5-dicarboxylate (300 mg, 1.24 mmol), Ser(OHn)-OMe-HCl salt (647 mg, 2.64 mmol), HOBt-H₂O (570 mg, 3.72 mmol) and EDC·HCl (690 mg, 3.60 mmol) in DMF (25 mL) was treated with TEA (2 mL). The resulting mixture was stirred for 16 h and concentrated. Work up as in Example 1 afforded 370 mg (51% yield) of the bisamide as a bright yellow powder: ¹H NMR (300 MHz, CDCl₃) δ 8.47 (d, J=8.7 Hz, 2H), 7.25-7.37 (complex m, 10H), 5.98 (bs, 4H), 4.85 (dt, J=8.7, 3.3 Hz, 2H), 4.56 (ABq, J=12.6, Hz, Δv=11.9 Hz, 4H), 3.99 (one half of an ABq of d, J=8.7, 3.3, Δv obscured, 2H), 3.76-3.80 (one half of an ABq-obsured, 2H), 3.78 (s, 6H), 13C NMR (75 MHz, CDCl₃) δ 170.5 (s), 161.5 (s), 146.8 (s), 138.7 (s) 128.6 (d), 128.1 (d), 127.8 (d), 126.9 (s), 73.5 (t), 69.8 (t), 53.0 (q), 52.9

To the product from step 2 (353 mg, 0.64 mmol) in methanol (20 mL) was added 5% Pd/C (300 mg) and ammonium formate (600 mg). The resulting reaction was heated at reflux for 2 h. The reaction was cooled to room temperature, filtered through a plug of celite and concentrated. The residue was recrystallized from methanol-ether to provide 191 mg (80% yield) of Example 3 as a yellow foam: ¹H NMR (300 MHz, DMSO-d₆) δ 8.48 (d, J=6.9 Hz, 2H), 6.72 (bs, 4H), 3.95 (apparent quartet, J=5.1 Hz, 2H), 3.60 (apparent ABq of doublet; down-field group centered at 3.71, J=9.9, 5.1 Hz, 2H; up-field group centered at 3.48, J=9.9, 6.3 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 172.9 (s), 164.9 (s), 147.0 (s), 127.0 (s), 62.9 (d), 55.7 (t). LCMS (5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention...
time = 1.45 min on 30 mm column, (M+H)^+ = 373. UV/vis (100 μM in PBS) \( \lambda_{\text{max}} = 343 \) nm. Fluorescence \( \lambda_{\text{exc}} = 449 \) nm, \( \lambda_{\text{em}} = 559 \) nm.

Example 4

3,6-bis(bis(2-methoxyethyl) amino)-N\(^2\),N\(^3\),N\(^4\),N\(^5\)-tetakis(2-methoxyethyl)pyrazine-2,5-dicarboxamide

Bis TFA Salt

Step 1. Synthesis of 3,6-dibromopyrazine-2,5-dicarboxylic acid

3,6-Diaminopyrazine-2,5-dicarboxylic acid (409 mg, 2.52 mmol) was dissolved in 48% hydrobromic acid (10 mL) and cooled to 0°C in an ice-salt bath. To this stirred mixture was added a solution of sodium nitrite (695 mg, 10.1 mmol) in water (10 mL) dropwise so that the temperature remains below 5°C. The resulting mixture was stirred for 3 h at 5-15°C, during which time the red mixture became a yellow solution. The yellow solution was poured into a solution of cupric bromide (2.23 g, 10.1 mmol) in water (100 mL) and the resulting mixture was stirred at room temperature. After an additional 3 h, the aqueous mixture was extracted with EtOAc (3×). The combined extracts were dried (Na\(_2\)SO\(_4\)), filtered, and concentrated to afford 440 mg (54% yield) 3,6-dibromopyrazine-2,5-dicarboxylic acid as a pale yellow solid. \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) δ 164.3 (s), 148.8 (s), 134.9 (s).

Step 2. Synthesis of 3-(Bis(2-methoxyethyl)amino)-6-bromo-N\(^2\),N\(^3\),N\(^4\),N\(^5\)-tetakis(2-methoxyethyl)pyrazine-2,5-dicarboxamide

HPLC (5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time = 2.95 min on 250 mm column.

Step 3

To the product from step 2 (116 mg, 0.19 mmol) was added bis(2-methoxyethyl)amine (3.0 mL, 2.71 g, 20.3 mmol) and a "spatula tip" of Pd(PPh\(_3\))\(_4\). The resulting mixture was heated to 140°C for 2 h. The reaction was cooled and concentrated. The residue was purified by flash chromatography (SiO\(_2\), 10/1 CHCl\(_3\)-MeOH). The resulting material was purified again by reverse phase medium pressure chromatography (C18, 10-50% manual gradient acetonitrile in 0.1% TFA) to afford 12 mg (10% yield) of Example 4 as an orange-brown film: LCMS (15-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time = 3.85 min on 250 mm
Example 5

3,6-diamino-N²,N⁵-bis(2-aminoethyl)pyrazine-2,5-dicarboxamide Bis TFA Salt

Step 1. Synthesis of 3,6-diamino-N²,N⁵-bis(2-(tert-butoxycarbonyl)aminoethyl)pyrazine-2,5-dicarboxamide

A mixture of sodium 3,6-diaminopyrazine-2,5-dicarboxylate (500 mg, 2.07 mmol), tert-butyl 2-aminoethylecarbamate (673 mg, 4.20 mmol), HOBT-H₂O (836 mg, 5.46 mmol) and EDC-HCl (1.05 g, 5.48 mmol) in DMF (25 mL) was stirred for 16 h and concentrated. Work up as in Example 1 afforded 770 mg (76% yield) of the bisamide as an orange foam:

^1^H NMR (300 MHz, DMSO-d₆) major conformation, δ 8.44 (t, J=5.7 Hz, 2H), 6.90 (t, J=5.7 Hz, 2H), 6.48 (bs, 4H), 2.93-3.16 (complex m, 8H), 1.37 (s, 9H), 1.36 (s, 9H). ^1^C NMR (75 MHz, DMSO-d₆), conformational isomers δ 165.1 (s), 155.5 (bs), 155.4 (bs), 146.0 (s), 126.2 (s), 77.7 (bs), 77.5 (bs), 45.2 (bt), 44.5 (bt), 28.2 (q).

Step 2

To the product from step 1 (770 mg, 1.60 mmol) in methylene chloride (100 mL) was added TFA (25 mL) and the reaction was stirred at room temperature for 2 h. The mixture was concentrated and the residue taken up into methanol (15 mL). Ether (200 mL) was added and the orange solid precipitate was isolated by filtration and dried at high vacuum to afford 627 mg (77% yield) of Example 5 as an orange powder:

^1^H NMR (300 MHz, DMSO-d₆) δ 8.70 (t, J=6 Hz, 2H), 7.86 (fs, 6H), 6.50 (bs, 4H), 3.46-3.58 (m, 4H), 3.26-3.40 (m, 4H). ^1^C NMR (75 MHz, DMSO-d₆) δ 166.4 (s), 146.8 (s), 127.0 (s), 39.4 (t), 37.4 (t). LCMS (5-95% gradient acetonitrile in 0.1% TEA over 10 min), single peak retention time=3.62 min on 30 mm column, (M+H)⁺=283. UV/vis (100 µM in PBS) λ_max=449 nm, λ_min=562 nm.

Example 6

3,6-Diamino-N²,N⁵-bis(D-Aspartate)-pyrazine-2,5-dicarboxamide

Step 1. Synthesis of 3,6-Diamino-N²,N⁵-bis(benzyl D-Obenzyl-Aspartate)-pyrazine-2,5-dicarboxamide

A mixture of sodium 3,6-diaminopyrazine-2,5-dicarboxylate (600 mg, 2.48 mmol), Asp(OBu)-OMe-p-TosH salt (2.43 g, 5.00 mmol), HOBT-H₂O (919 mg, 6.00 mmol) and EDC-HCl (1.14 g, 5.95 mmol) in DMF (50 mL) was treated with TEA (4 mL). The resulting mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the residue was partitioned with water and EtOAc. The EtOAc layer was separated and washed successively with saturated sodium bicarbonate, water and brine. The EtOAc solution was dried (Na₂SO₄), filtered and concentrated. The residue was purified by flash chromatography (SiO₂, 50/1 CHCl₃-MeOH to 10/1) to afford 1.15 g of the bis-amide (58% yield) as a yellow foam:

^1^H NMR (500 MHz, CDCl₃) δ 8.61 (d, J=8.4 Hz, 2H), 7.29-7.39 (m, 20H), 5.85 (bs, 4H), 5.22 (ABq, J=10.0 Hz, Δv=17.3 Hz, 4H), 5.10 (ABq, J=12.2 Hz, Δv=34.3 Hz, 4H), 5.06-5.09 (obs m, 2H), 3.11 (ABq of d, J=17.0, 5.14 Hz, 2H).
Hz, δ=77.9 Hz, 4H). 13C NMR (75 MHz, CDCl3) δ 170.7 (s), 170.7 (s), 165.4 (s), 147.0 (s), 135.7 (s), 135.6 (s), 129.0 (d), 128.9 (d), 128.8 (d), 128.7 (d), 128.7 (d), 126.9 (s), 68.0 (t), 67.3 (t), 49.1 (d), 37.0 (t). LCMS (50-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=5.97 min on 250 mm column, (M+H)+=789.

Step 2

To the product from step 1 (510 mg, 0.65 mmol) was added THF (20 mL) and water (10 mL). The stirred mixture was added 10% Pd/C (500 mg) and ammonium formate (1 g). The resulting mixture was heated to 60°C for 2 h and allowed to cool to room temperature. The mixture was filtered through celite and concentrated. The resulting material was purified again by reverse phase medium pressure chromatography (C18, 10-70% manual gradient acetonitrile in 0.1% TFA) to afford 137.8 mg (54% yield) of Example 6 as an orange solid:

1H NMR (300 MHz, DMSO-d6) δ 8.62 (d, J=8.4 Hz, 2H), 6.67 (bs, 4H), 4.725 (dt, J=8.4, 5.4 Hz, 2H), 2.74-2.88 (complex m, 4H). 13C NMR (75 MHz, DMSO-d6) δ 172.6 (s), 165.2 (s), 137.6 (s), 126.6 (s), 60.8 (t), 49.1 (d). LCMS (5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=4.01 min on 250 mm column, (M+H)+=429, UV/vis (100 μM in PBS) λmax=433 nm. Fluorescence (100 nM) λex=449 nm, λem=558 nm.

Example 7

3,6-Diamino-N3,N5-bis[14-oxa-2,5,8,11-tetraoxa-15-azahptadecan-17-yl]pyrazine-2,5-dicarboxamide

To a solution of Example 5 (77.4 mg, 0.15 mmol) in DMF (5 mL) was added TEA (151 mg, 1.49 mmol) and 2,5-dioxopyrrolidin-1-yl 2,5,8,11-tetraoxatetradecan-14-oate (115 mg, 0.34 mmol) and the reaction was stirred for 16 h at room temperature. The reaction was concentrated and the residue was purified by medium pressure reversed phase chromatography (LiChroprep RP-18 Lobar (B) 25×310 mm—EM chemicals 40-63 μm, ~70 g, 90/10 to 80/20 0.1% TFA-ACN) to afford 37.4 mg (35% yield) of example 7 as an orange film:

1H NMR (300 MHz, DMSO-d6) δ 8.47 (t, J=5.7 Hz, 2H), 7.96 (t, J=5.4 Hz, 2H), 3.20-3.60 (complex m, 3H), 3.47 (s, 3H), 3.46 (s, 3H), 2.30 (t, J=6.3 Hz, 4H). 13C NMR (75 MHz, DMSO-d6) δ 170.2 (s), 165.1 (s), 146.0 (s), 126.2 (s), 71.2 (t), 69.7 (t), 69.6 (t), 69.5 (t), 69.4 (t), 66.7 (t), 58.0 (q), 38.2 (t), 36.2 (t). LCMS (5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=4.01 min on 250 mm column, (M+H)+=719, (M+Na)+=741. UV/vis (100 μM in PBS) λmax=437 nm. Fluorescence (100 nM) λex=437 nm, λem=559 nm.

Example 8

3,6-Diamino-N3,N5-bis[26-oxa-2,5,8,11,14,17,20,23-octaoxa-27-azanonacosen-29-yl]pyrazine-2,5-dicarboxamide
To a solution of Example 5 (53.1 mg, 0.10 mmol) in DMF (5 mL) was added TEA (114 mg, 1.13 mmol) and 2,5-dioxopyrrolidin-1-yl 2,5,8,11,14,17,20,23-octaoxahexacosan-26-oate (128 mg, 0.25 mmol) and the reaction was stirred for 16 h at room temperature. The reaction was concentrated and the residue was purified by medium pressure reversed phase chromatography (LiChroprep RP-18 Lobar (B) 25×310 mm—EMD chemicals 40-63 μm, ~70 g, 90/10 to 80/200.1% TFA-ACN) to afford 87.5 mg (82% yield) of example 8 as an orange film: 1H NMR (300 MHz, DMSO-d6) δ 8.46 (t, J=5.7 Hz, 2H), 7.96 (t, J=5.4 Hz, 2H), 3.16-3.73 (complex m, 7H), 2.28-2.32 (m, 2H). 13C NMR (75 MHz, DMSO-d6)—multiple conformations—δ 170.1 (s), 169.9 (s) 169.8 (s), 165.1 (s), 146.0 (s), 126.2 (s), 71.2 (t), 69.7 (t), 69.6 (t), 69.5 (t), 66.7 (t), 58.0 (q), 38.2 (t), 36.2 (t). LCMS (15-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time: 5.90 min on 250 mm column, (M+H)+ = 410.2, (M+H)+ = 536. UV/vis (100 μM in PBS) λmax = 438 nm. Fluorescence (100 nM) λex = 438 nm, λem = 560 nm.

Example 9

3,6-Diamino-N2,N5-bis-(38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxacostriacontan-38-oate (144 mg, 0.21 mmol) in DMF (2.0 mL) and the resulting mixture was stirred for 16 h thereafter. The reaction was concentrated and the residue was purified by medium pressure reversed phase chromatography (LiChroprep RP-18 Lobar (B) 25×310 mm—EMD chemicals 40-63 μm, ~70 g, 90/10 to 80/20 0.1% TFA-ACN) to afford 87.5 mg (61% yield) of example 9 as an orange film: 1H NMR (300 MHz, DMSO-d6) δ 8.48 (t, J=5.7 Hz, 2H), 7.96 (t, J=5.4 Hz, 2H), 7.80-7.86 (m, 2H), 5.94 (bm, 2H), 3.30-3.60 (complex m, 106H), 2.26-2.33 (m, 4H). 13C NMR (75 MHz, DMSO-d6) δ 170.2 (s), 165.1 (s), 146.0 (s), 126.2 (s), 71.2 (t), 69.7 (t), 69.6 (t), 69.5 (t), 66.7 (t), 58.0 (q), 38.2 (t), 36.2 (t). LCMS (15-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time: 5.90 min on 250 mm column, (M+H)+ = 559. UV/vis (100 μM in PBS) λmax = 449 nm. Fluorescence (100 nM) λex = 449 nm, λem = 559 nm.
Example 10

Bis(2-(PEG-5000)ethyl) 6-(2-(3,6-diamino-5-(2-aminoethylearboxamyl)pyrazine-2-carboxamido)ethylamino)-6-oxohexane-1,5-diyllicarbamate

Overall MW ~ 11,000

A solution of Example 5 (25 mg, 0.049 mmol) in DMF (30 mL) was treated with TEA (1 mL) and m-PEG2-NHS (1 g, 0.1 mmol) and the resulting mixture was stirred for 48 h at room temperature. The mixture was concentrated and the residue was partially purified by gel filtration chromatography (G-25 resin, water). The extract was concentrated and further purified by reverse phase medium pressure chromatography (C18, 10-70% acetonitrile in 0.1% TFA) to afford 137.8 mg (54% yield) of Example 10 as a tan waxy solid: MALDI MS m/z=11393.

Example 11

(R)-2-(6-(bis(2-methoxyethyl)amino)-5-cyano-3-morpholinopyrazine-2-carboxamido) succinic Acid

Step 1. Synthesis of 2-amino-5-bromo-3,6-dichloropyrazine

A solution of 2-amino-6-chloropyrazine (25 g, 193.1 mmol) in MeOH (500 mL) was treated with NBS (34.3 g, 193.1 mmol), portion-wise, over 1 hour. The resulting mixture was stirred for 16 hours thereafter. TLC analysis at this time shows a small amount of starting material remaining. Another 1.4 g NBS added and reaction heated to 50° C for 2 hours. The mixture was then cooled to 38° C, and treated with NCS (25.8 g, 193.1 mmol). The reaction mixture was heated to 50° C, for 16 hours thereafter. The mixture was then cooled to room temperature and treated with water (500 mL). The precipitate was collected by filtration and dried in a vacuum desiccator to afford 45.4 g (97% yield) of 2-amino-5-bromo-3,6-dichloropyrazine as a white solid: 13C NMR (75 MHz, CDCl3) δ 149.9 (s), 145.6 (s), 129.6 (s), 121.5 (s). LCMS (15-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=4.51 min on 30 mm column, (M+H)+=244, (M+H+ACN=)+=285.

Step 2. Synthesis of 5-amino-3,6-dichloropyrazine-2-carbonitrile

A mixture of CuCN (8.62 g, 96.3 mmol) and NaCN (4.72 g, 96.3 mmol) was heated under high vacuum to 90° C. The
resulting mixture was subjected to three Argon/Vacuum cycles and placed under a final positive pressure of Argon. The mixture was allowed to cool to room temperature and DMF (150 mL) was added. The heterogeneous mixture was heated to 130°C for 2.5 hours. To the resulting homogeneous mixture of sodium dicyanocuprate was added a solution of the product from step 1 (15.6 g, 64.2 mmol) dissolved in DMF (150 mL), dropwise, over 1 hour. The temperature was gradually raised to 150°C, and the resulting mixture was stirred at this temperature for 10 hours thereafter. The reaction was then allowed to cool to room temperature and poured into water (1 L). The resulting mixture was extracted with EtOAc (3x) and the combined extracts were filtered to remove a flocculant dark solid, washed with brine, dried (Na₂SO₄), filtered again and concentrated. Purification by flash column chromatography (SiO₂ 10/1 hexanes-EtOAc to 3/1) to afford 6.70 g (55% yield) of the nitrile product as a tan solid. 

^{13}C NMR (75 MHz, CDCl₃) δ 153.9 (s), 149.1 (s), 131.7 (s), 115.4 (s), 111.0 (s). GCMS (m/z): temperature 280°C, 1.0 mL/min helium flow rate, temperature program: 100°C (2 min hold), ramp to 300°C at 10°C/min (2 min hold), major peak retention time = 16.556 min, m/z (EI) = 188, 190.

Step 3. Synthesis of 5-amino-3-(bis(2-methoxyethyl)amino)-6-chloropyrazine-2-carbonitrile

To the product from step 2 (1.00 g, 5.29 mmol) in ACN (20 mL) was added bis(2-methoxyethyl)amine (3.0 mL, 2.71 g, 20.3 mmol) and the reaction mixture was heated to 70°C for 16 hours thereafter. The reaction was cooled and concentrated. The residue was partitioned with EtOAc and water. The organic layer was separated and the aqueous was extracted again with EtOAc. The combined organic extracts were washed with brine, dried (Na₂SO₄), filtered and concentrated. Purification by flash column chromatography (SiO₂ 10/1 hexanes-EtOAc to 1/1) afforded 950 mg (63% yield) of the desired adduct as a yellow solid. 

^{1}H NMR (300 MHz, CDCl₃) δ 7.47 (bs, 2H), 3.77 (t, J=5.7 Hz, 4H), 3.52 (t, J=5.4 Hz, 4H), 3.25 (s, 6H). 

To the product from step 3 (1.39 g, 4.88 mmol) in 48% hydrobromic acid (20 mL) at 0°C (ice-salt bath), was added a solution of sodium nitrite (673 mg, 9.75 mmol) in water (10 mL) dropwise over 30 min. The resulting mixture was stirred at 0–5°C for 1 h and poured into a stirred solution of CuBr₂ (1.64 g, 7.34 mmol) in water (100 mL). The resulting mixture was stirred for 16 h at room temperature thereafter. The mixture was extracted with EtOAc (3x). The combined organic layers were dried (Na₂SO₄), filtered and concentrated. Purification by flash column chromatography (SiO₂ 50/1 CHCl₃-MeOH) afforded 1.00 g (58% yield) of the bromide as an orange-brown solid. 

^{1}H NMR (300 MHz, CDCl₃) δ 3.99 (t, J=5.4 Hz, 4H), 3.64 (t, J=5.4 Hz, 4H), 3.35 (s, 6H). 

To the product from step 4 (1.0 g, 2.87 mmol), 2-furanboronic acid (643 mg, 5.75 mmol), Cs₂CO₃ (3.31 g, 10.2 mmol), TTP (35 mol %, 236 mg, 1.02 mmol), and Pd₂dba₃·CHCl₃ (5 mol %, 10 mol % Pd, 150 mg) was subjected to 3 vacuum/Argon cycles and placed under a positive pressure of Argon. Anhydrous dioxane (50 mL) was added and the reaction mixture was heated to 75°C for 16 h thereafter. The reaction mixture was cooled to room temperature, diluted with EtOAc (100 mL) and filtered through a medium iris. Concentration and purification of the residue by flash chromatography (SiO₂ 50/1 CHCl₃-MeOH) afforded the 757 mg of the furan adduct (78% yield) as a tan powder.
US 8,722,685 B2

29

(5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=6.41 min on 250 mm column, (M+H)^+=337.

Step 6. Synthesis of 6-(bis(2-methoxyethyl)amino)-3-chloro-5-cyanopyrazine-2-carboxylic Acid

To a well stirred mixture of ACN (11 mL), CCl4 (7 mL), and water (11 mL) were added sodium periodate (1.07 g, 5.00 mmol) and Rut2H2O (13.3 mg, 0.10 mmol), sequentially. The resulting mixture was stirred vigorously at room temperature for 30 min and treated with sodium bicarbonate (2.10 g, 25.0 mmol) followed by water (5 mL). Vigorous stirring for another 15 minutes was followed by the addition of a solution of the product from Step 5 (276 mg, 0.82 mmol) dissolved in ACN (1 mL). The green mixture was stirred at room temperature for 5.5 h. The mixture was transferred to a separatory funnel and extracted with EtOAc. The aqueous layer was adjusted to pH~3.5 and extracted again with EtOAc (2x). The combined extracts were washed with 20% sodium bisulfite and brine and dried (Na2SO4). Filtration and concentration afforded 140 mg (54% yield) of carboxylic acid as a pale yellow solid. LCMS (5-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=5.05 min on 250 mm column, (M+H)^+=315.

30

Step 7. Synthesis of (R)-dibenzy1 2-(6-(bis(2-methoxyethyl)amino)-3-chloro-5-cyanopyrazine-2-carboxamido)succinate

To the product from step 7 (240 mg, 0.39 mmol) was added morpholine (5 mL). The reaction mixture was heated to 70°C for 2 h. The mixture was cooled and concentrated. The residue was partitioned with EtOAc and water. The EtOAc layer was separated and washed with saturated sodium bicarbonate and brine. The EtOAc layer was dried (Na2SO4), filtered and concentrated. Purification by flash column chromatography (SiO2, 3:1 to 1:1 hexanes-EtOAc) afforded 199 mg (75% yield) of the morpholine adduct as an orange foam. LCMS (15-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=8.76 min on 250 mm column, (M+H)^+=661, (M+Na)^+=683.

Step 8. (R)-dibenzy1 2-(6-(bis(2-methoxyethyl)amino)-5-cyano-3-morpholinopyrazine-2-carboxamido)succinate

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A mixture of the product from step 6 (140 mg, 0.45 mmol), EDC.HCl (128 mg, 0.67 mmol) and HOBr.H2O (102 mg, 0.67 mmol) in anhydrous DMF (25 mL) was stirred together at room temperature for 30 min. To this stirred mixture was added (R)-dibenzy1 2-amino succinate p-TsOH salt (213 mg, 0.44 mmol) followed by TEA (1 mL). The resulting mixture was stirred for 16 h thereafter. The reaction mixture was concentrated and partitioned with EtOAc and saturated sodium bicarbonate solution. The EtOAc layer was separated

and washed with saturated sodium bicarbonate and brine, dried (Na2SO4), filtered and concentrated to afford 240 mg (88% yield) of the pyrazine amide as an orange foam. LCMS (15-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=8.76 min on 250 mm column, (M+H)^+=610, (M+Na)^+=632.

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The dibenzy1 ester (115 mg, 0.17 mmol) in THF (10 mL) was added 1.0 N sodium hydroxide (4 mL). The mixture was stirred for 1 h at room temperature. The pH was adjusted to ~2 with 1.0 N HCl and the solution was concentrated. Purification of the residue by medium pressure reversed phase chromatography (Lichrep RP-18 Lobar (B) 25x310 mm—EMD chemicals 40-63 μm, -70 g, 90/10 to 50/50 0.1% TFA-ACN) afforded 32 mg (27% yield) of example 11 as an
orange solid: LCMS (15-95% gradient acetonitrile in 0.1% TFA over 10 min), single peak retention time=4.47 min on 250 mm column, (M+H)+=481. UV/vis (100 µM in PBS) λ_{max}=438 nm. Fluorescence (100 nM) λ_{exc}=449 nm, λ_{em}=570 nm.

Example 12

Protocol for Assessing Renal Function

An example of an in vivo renal monitoring assembly 10 is shown in FIG. 2 and includes a light source 12 and a data processing system 14. The light source 12 generally includes or is interconnected with an appropriate device for exposing at least a portion of a patient’s body to light therefrom. Examples of appropriate devices that may be interconnected with or be a part of the light source 12 include, but are not limited to, catheters, endoscopes, fiber optics, ear clips, hand bands, head bands, forehead sensors, surface coils, and finger probes. Indeed, any of a number of devices capable of emitting visible and/or near infrared light of the light source may be employed in the renal monitoring assembly 10.

Still referring to FIG. 2, the data processing system 14 of the renal monitoring assembly 10 may be any appropriate system capable of detecting spectral energy and processing data indicative of the spectral energy. For instance, the data processing system 14 may include one or more lenses (e.g., to direct and/or focus spectral energy), one or more filters (e.g., to filter out undesired wavelengths of spectral energy), a photodiode (e.g., to collect the spectral energy and convert the same into electrical signal indicative of the detected spectral energy), an amplifier (e.g., to amplify electrical signal from the photodiode), and a processing unit (e.g., to process the electrical signal from the photodiode). This data processing system 14 is preferably configured to manipulate collected spectral data and generate an intensity/time profile and/or a concentration/time curve indicative of renal clearance of a pyrazine derivative of the present invention from the patient 20. Indeed, the data processing system 14 may be configured to generate appropriate renal function data by comparing differences in manners in which normal and impaired cells remove the pyrazine derivative from the bloodstream, to determine a rate or an accumulation of the pyrazine derivative in organs or tissues of the patient 20, and/or to provide tomographic images of organs or tissues having the pyrazine derivative associated therewith.

In one protocol for determining renal function, an effective amount of a pyrazine derivative of the invention is administered to the patient (e.g., in the form for a pharmaceutically acceptable composition). At least a portion of the body of the patient 20 is exposed to visible and/or near infrared light from the light source 12 as indicated by arrow 16. For instance, the light from the light source 12 may be delivered via a fiber optic that is affixed to an ear of the patient 20. The patient may be exposed to the light from the light source 12 before or after administration of the pyrazine derivative to the patient 20. In some cases, it may be beneficial to generate a background or baseline reading of light being emitted from the body of the patient 20 (due to exposure to the light from the light source 12) before administering the pyrazine derivative to the patient 20. When the pyrazine derivative that is in the body of the patient 20 is exposed to the light from the light source 12, the pyrazine derivative emanates light (indicated by arrow 18) that is detected/colllected by the data processing system 14. Initially, administration of the pyrazine derivative to the patient 20 generally enables an initial spectral signal indicative of the initial content of the pyrazine derivative in the patient 20. The spectral signal then tends to decay as a function of time as the pyrazine derivative is cleared from the patient 20. This decay in the spectral signal as a function of time is indicative of the patient’s renal function. For example, in a first patient exhibiting healthy/normal renal function, the spectral signal may decay back to a baseline in a time of T1. However, a spectral signal indicative of a second patient exhibiting deficient renal function may decay back to a baseline in a time of T4+4 hours. As such, the patient 20 may be exposed to the light from the light source 12 for any amount of time appropriate for providing the desired renal function data. Likewise, the data processing system 14 may be allowed to collect/detect spectral energy for any amount of time appropriate for providing the desired renal function data.

REFERENCES


What is claimed is:
1. A compound of Formula I, wherein:

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\text{Formula I}
\]

each of X1 and X2 is independently —CN, —CO2R1, —CONH2R3, or —CONH(PS);

each of Y1 and Y2 is independently —NR2R5 or

\[
\text{Z1}
\]

Z1 is a single bond, —CR18R19—, —O—, —NR20—, —NOR21—, —S—, —SO—, or —SO2—;

each of R5 to R26 and R40 to R42 is independently —H, —CH2(CHOH)R44, —CH2(CHOH)2CO2H, —(CH2)2CO2H, —(CH2)2SO2H, —(CH2)2SO3H, —(CH2)2SO4H, —(CH2)2SO4H, and

PS is a sulfated or non-sulfated polysaccharide chain comprising one or more monosaccharide units connected by glycosidic linkages; and

‘a’ is an integer from 1 to 10, ‘c’ is an integer from 1 to 100, and each of ‘m’ and ‘n’ independently is an integer from 1 to 3;

with the proviso that if:

each of X1 and X2 is independently —CN, —CO2R1 or —CONH2R3;
each of $Y^1$ and $Y^2$ is independently $-NR^4R^5$ or

$$\begin{array}{c}
\text{N} \\
\text{(CH}_2\text{)}_{2m} \\
\text{(CH}_2\text{)}_{2n} \\

\end{array}$$

and

$Z^1$ is a direct bond, then:

each of $R^1$, $R^2$, $R^3$, $R^4$ and $R^5$ is not simultaneously $-H$;
and

$m$, $n$, $N$ and $Z^1$ together do not form a 5- or 6-membered ring.

2. The compound of claim 1, wherein each of $X^1$ and $X^2$ is independently $-CONR^2R^3$, and each of $Y^1$ and $Y^2$ is independently $-NR^4R^5$.

3. The compound of claim 2, wherein each of $R^2$ and $R^4$ is $-H$, and $R^3$ is $CH_2(CHOH)R^{44}$ or $-(CH_2CH_2O)_cR^{45}$, and $R^5$ is $-H$ or $-(CH_2CH_2O)_cR^{46}$.

4. The compound of claim 3, wherein $R^3$ is $-(CH_2CH_2O)_cR^{44}$, and $R^5$ is $-H$.

5. The compound of claim 4, wherein $R^{45}$ is $-CH_3$, and $c'$ is an integer from 1 to 20.

6. The compound of claim 1, wherein each of $X^1$ and $X^2$ is independently $-CO_2R^1$, and each of $Y^1$ and $Y^2$ is independently $-NR^4R^5$.

7. The compound of claim 6, wherein each of $R^1$ and $R^4$ is $-H$, and $R^5$ is $-CH_2(CHOH)R^{44}$ or $-(CH_2CH_2O)_cR^{45}$.

8. The compound of claim 7, wherein $R^{44}$ is $-H$, $R^{45}$ is $-CH_3$, $a'$ is an integer from 1 to 6, and $c'$ is an integer from 1 to 20.

9. The compound of claim 1, wherein each of $X^1$ and $X^2$ is $-CN$, and each of $Y^1$ and $Y^2$ is $-NR^4R^5$.

10. The compound of claim 9, wherein $R^4$ is $-H$, and $R^5$ is $-CH_2(CHOH)R^{44}$ or $-(CH_2CH_2O)_cR^{45}$.

11. The compound of claim 10, wherein $R^{44}$ is $-H$, $R^{45}$ is $-CH_3$, $a'$ is an integer from 1 to 6, and $c'$ is an integer from 1 to 20.

12. The compound of claim 1, wherein each of $X^1$ and $X^2$ is $-CONH(PS)$, and each of $Y^1$ and $Y^2$ is $-NR^4R^5$.

13. The compound of claim 12, wherein each of $R^4$ and $R^5$ is independently $-H$ or $-CH_2(CHOH)R^{44}$.

14. The compound of claim 13, wherein each of $R^4$ and $R^5$ is $-H$.

15. The compound of claim 12, wherein the one or more monosaccharide units of the polysaccharide chain (PS) are selected from the group consisting of glucose, fructose, mannose, xylose, and ribose.

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