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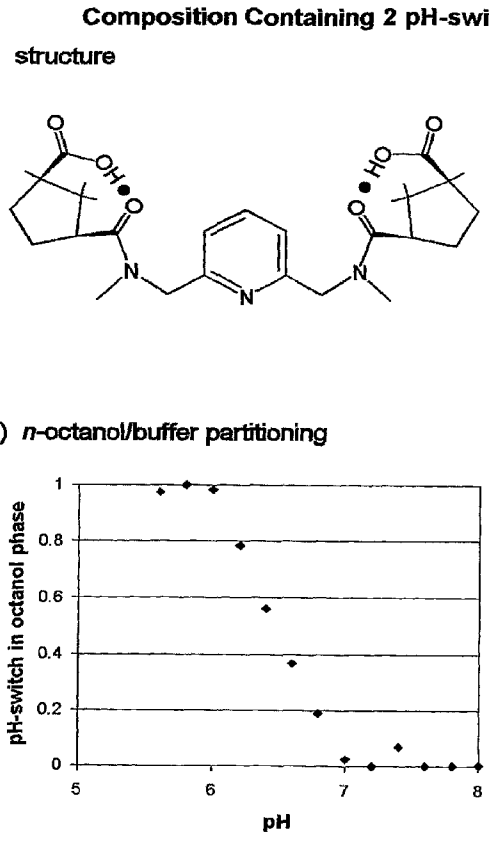
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(54) Title: ONCO-TOOL COMPOSITIONS AND METHODS OF USE FOR DETECTING AND TREATING TUMORS



(57) Abstract: One of the most universal characteristics of malignant tumors is their acidity. Onco-tools are small non-peptide synthetic molecules designed to exploit this acidity for early detection and destruction of tumors. Each onco-tool has a structure which is anionic and hydrophilic at pH 7.4 and so repels from the negatively-charged surfaces of cells in normal tissues. When an onco-tool enters an acidic environment, such as in a tumor, a portion of the onco-tool molecules switch to their non-ionic lipophilic form which is designed to enter cells, such as cells in acidic areas of tumors. Prior to use of an onco-tool, a selected radioisotope is linked to the onco-tool. If that radioisotope emits radiation which can be detected outside the body, then the onco-tool can serve for detecting tumors. If that radioisotope emits radiation effective to kill cells, then the onco-tool can serve for treating tumors.

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07SUMPCT01

ONCO-TOOL COMPOSITIONS AND METHODS OF USE FOR DETECTING AND TREATING TUMORS

RELATED PATENT APPLICATIONS & INCORPORATION BY REFERENCE:

This application is a PCT application filed pursuant 35 USC 363 and claims priority based on U.S. Utility Patent Application No. 11/395,487, filed March 30, 2006, and U.S. Utility Patent Application Nos. 11/449,495 and 11/449,508, both filed on June 7, 2006.

These related utility patent applications are incorporated herein by reference and made a part of this application. If any conflict arises between the disclosure of the invention in this PCT application and that in the related utility patent applications, the disclosure in this PCT application shall govern. Moreover, any and all U.S. patents, U.S. patent applications, and other documents, hard copy or electronic, cited or referred to in this application are incorporated herein by reference and made a part of this application.

TECHNICAL FIELD:

This invention relates to compositions effective to be selectively sequestered in acidic areas of tumors within a subject for the purpose of detecting and treating tumors.

BACKGROUND OF THE INVENTION:

Acidity: an exploitable near-universal characteristic of tumors:

In 1930 the famous physiologist Otto Warburg reported that one of the most universal characteristics of malignant tumors is their acidity. Subsequent research suggests that such acidity arises because for a tumor to grow larger

than about 1 millimeter in diameter it must induce new blood vessels. Because tumor-induced blood vessels are poorly spaced and abnormal, areas of tumors more than a few tens of microns away from such capillaries generally become hypoxic (oxygen deficient). Cells in such hypoxic areas of tumors either die or switch to glycolytic metabolism - resulting in their excreting lactic acid. Because of the poor circulation in a tumor, that excreted lactic acid builds up in the interstitial space between cells in the hypoxic areas of the tumor. This can result in a pH as low as 6.0 in areas most distant from capillaries, up to about pH 7.0 close to capillaries. For comparison, the pH in the interstitial space in normal tissues is tightly regulated between about pH 7.3 and pH 7.5.

Quiescent cells in acidic areas of tumors: the great unmet challenge in cancer therapy:

As noted above, the acidity characteristic of tumors generally increases with increasing distance from tumor capillaries. While tumor cells at near-normal pH in close proximity to capillaries have high metabolic rates and fast cell division, those tumor cells in more acidic areas at a greater distance from capillaries have lower metabolic rates and divide slowly or not at all. Compared to the fast-dividing tumor cells, these slow-dividing and non-dividing tumor cells, referred to as quiescent, are substantially more resistant to cell-damaging agents, such as radiation and toxic chemicals.

Since conventional cancer therapies (radiation and chemotherapy) were selected in large part for their ability to kill rapidly-dividing cells while sparing the slow-dividing and non-dividing cells typical of most normal tissues, such conventional cancer therapies are fairly effective in killing the rapidly-dividing tumor cells at near-normal pH close to capillaries. But not surprisingly, those same therapies are rather ineffective against the slow-dividing and non-dividing quiescent cells in more acidic areas of a tumor. Consequently, conventional cancer treatments predominantly kill the well-oxygenated fast-dividing tumor cells while sparing the quiescent cells in hypoxic/acidic areas of that tumor. This initial killing of the fast-dividing cells causes the tumor to go into remission while those killed cells are disposed of by the body's normal cleanup processes. However, during the course of this cleanup process, all too often the surviving treatment-resistant quiescent cells in the acidic areas of the tumor slowly regain access to

adequate oxygen, nutrients, and waste disposal - eventually allowing them to revert to high metabolic rate and fast cell division. This rejuvenation of the previously-quiescent tumor cells commonly leads to the dreaded relapse that is responsible for most deaths from cancer.

Prior efforts to exploit hypoxic/acidic areas of tumors for therapy:

The hypoxic/acidic properties of tumors have been known for over 75 years and it has long been speculated that such properties might be exploitable for therapy. However, until recently the most successful efforts to exploit these properties have focused on the hypoxia. Specifically, substances have been developed which exhibit minimal cytotoxicity in normoxic cells, while exhibiting considerable cytotoxicity in hypoxic cells. One such agent has progressed to the clinical trials stage.

Compared to exploiting the hypoxia in tumors, there has been much less effort focused on exploiting the acidity of tumors. One unsuccessful approach was based on the observation that acid pH in tissues acts to sensitize those tissues to thermal damage. However, efforts to exploit this acid-mediated thermal sensitivity of cells gave disappointing results.

Another approach relating to acidity in tumors is based on the fact that the low pH in tumors ionizes weak-base cytotoxic agents and thereby renders them membrane-impermeable, which in turn results in preferential reduction of entry of a number of such weak-base agents into acidic areas of tumors relative to entry of such agents into cells in areas of more normal pH. In this regard, rather than attempting to exploit the low pH in the tumor, efforts were instead focused on raising the pH in the tumors as a means to partially de-ionize and thereby enhance the entry of such weak-base cytotoxic agents into cells of the tumor. Such efforts have met with some success.

Still another approach that relates more closely to the present invention relied on the fact that the low pH in the interstitial space of tumors will cause partial de-ionization of the weak-acid cytotoxic agent, Chlorambucil (shown in Figure 1). As a consequence of the lesser degree of ionization of this well known weak-acid cytotoxic drug in acidic areas of tumors, one would expect that it should show enhanced entry and hence greater cytotoxicity in cells in acidic areas of tumors. This expectation has been tested by Kozin et al., wherein they

used established methods to make tumors in tumor-bearing mice more acidic (Cancer Research Vol. 61, pages 4740 - 4743 (2001)). They reported that, as predicted, a reduction of about 0.3 pH units in the tumors coincided with a modest 1.7-fold improvement in tumor growth delay afforded by the weak-acid (pKa 5.8) Chlorambucil. In the conclusion to their paper they wrote: "To our knowledge, CHL (Chlorambucil) is the only clinical therapeutic that is a weak-acid with the appropriate pKa $\leq$ 6.5. This study thus provides a rationale for the design of novel, potent drugs exhibiting similar weak-acid properties and for which diffusion contributes to intracellular uptake. As also shown here, the combined use of such compounds with radiation and/or modulators of the pH gradient provide additional opportunities for maximizing the therapeutic response."

In the aforementioned paper the authors noted that their results showing modest enhancement of the effectiveness of the off-the-shelf cytotoxic drug Chlorambucil provided a rationale for designing new weak-acid cytotoxic anti-cancer drugs which may show high efficacy – but no guidance was given on prospective molecular structures of such drugs, nor was any guidance given on how to go about integrating both the weak acid function and the cytotoxic function into a suitable drug structure, nor was any guidance given on what specific properties are desirable, nor was any guidance given on how to go about designing, preparing, or testing such weak-acid cytotoxic drugs, nor was any guidance given concerning applications or methods of use of such cytotoxic drugs. A more serious limitation in the authors' proposal that novel weak-acid cytotoxic drugs of improved potency could be designed based on the Chlorambucil model is that the quiescent cells in acidic areas of tumors, which such drugs are designed to preferentially enter, are particularly resistant to conventional cytotoxic drugs by virtue of those cells being quiescent. Thus, a strong case can be made that conventional cytotoxic drugs, such as Chlorambucil, are inadequate for killing the quiescent cells in acidic areas of tumors for which they have been proposed to be used.

Instead, Summerton (inventor of onco-tools) contends that what is required for effectively destroying quiescent cells in acidic areas of tumors are specially-designed drugs which have a particularly devastating impact on cell viability sufficient to decisively destroy those treatment-resistant quiescent tumor

cells. Coupled with this requirement for a devastating impact on cell viability is the co-requirement that such drugs be effectively delivered into cells of the tumor while being largely excluded from cells in normal tissues.

BACKGROUND ART:

In contrast to the proposal of Kozin et al, to use conventional weak-acid cytotoxic drugs to exploit the acidity of tumors, a number of years ago Dr. James Summerton, the inventor of onco-tools, began the development of novel peptide compositions explicitly designed to exploit the pH differential between normal tissues and acidic regions of tumors, with the objective of providing safer and more effective treatments for a broad range of tumors. Those large peptide compositions (typically with masses in the range of 2,000 to 4,000 daltons), are referred to as "embedder and transporter peptides". Their structures and methods of use for detecting and treating tumors are disclosed in pending US Patent Application No. 11/069,849 and in US Patent No. 7,132,393 issued Nov. 7, 2006 – Dr. James E. Summerton the inventor of both. While such embedder and transporter peptides have been found to enter tumors in tumor-bearing mice with fair selectivity, nonetheless, experimental results from mouse studies suggest that such peptides achieve less specificity and efficacy than desired. They also appear to be subject to undue re-uptake in the kidneys - which is undesirable in diagnostic applications, and unacceptable in therapeutic applications.

In view of the limitations of the embedder and transporter peptides, Summerton subsequently embarked on a quest to devise an entirely new class of small non-peptide drugs, called onco-tools, with the objective of achieving considerably improved specificity, efficacy, and safety relative to the earlier embedder and transporter peptides. These small non-peptide onco-tools comprising the present invention have been disclosed by the present inventor, Dr. James E. Summerton, only in pending US Patent Application No. 11/395,487 filed on March 30, 2006, and US Patent Application Nos. 11/449,495 and 11/449,508 both filed on June 7, 2006.

SUMMARY OF THE INVENTION:

The object of the present invention is to provide non-peptide compositions and methods for detecting and treating tumors containing acidic areas.

Onco-tools exploit the acidity of tumors for early detection and destruction of tumors:

Onco-tools of the present invention are a novel class of molecules designed for detecting and treating tumors. Each onco-tool contains two or more pH-switch components which readily convert between an anionic hydrophilic form at a higher pH and a non-ionic lipophilic form at a lower pH. Each onco-tool also contains a cargo component which is effective to bind a selected radioisotope which is suitable for carrying out the diagnostic or therapeutic role of the onco-tool. Onco-tools consist of relatively small non-peptide synthetic molecules with masses typically in the range of about 300 to 1500 daltons - not counting the mass of the radioisotope.

At the neutral pH of normal tissues, onco-tools exist in their water-soluble negatively-charged form which is designed to be repelled from the negatively-charged surfaces of cells and to be readily excreted by the kidneys. Conversely, when onco-tools enter an acidic region of a tumor, a portion of the onco-tool molecules switch to their non-ionic lipid-soluble form which is designed to rapidly enter any nearby cell. Thus, when a dose of onco-tool is injected into a person, if that person has a tumor larger than approximately 1 millimeter in diameter, a portion of the injected dose is designed to rapidly enter and remain within cells in acidic regions of the tumor, with the remainder of the dose being excreted by the kidneys. Stated differently, onco-tools are designed to have the key properties of: a) being repelled from cells in normal tissues; b) being sequestered within acidic areas of tumors; and, c) any onco-tool not sequestered in an acidic area of a tumor is designed to be rapidly cleared from the body via the kidneys.

Prior to use of an onco-tool a selected radioisotope is linked to that onco-tool. That attached radioisotope carries out the onco-tool's diagnostic or therapeutic role. The diagnostic role is to report the onco-tool's presence within a tumor to a detector outside the body. The therapeutic role is to destroy the

tumor.

Diagnostic onco-tools:

Because of the great variability between tumors, routine detection of a wide range of tumor types at a sufficiently early stage that they can be successfully treated (preferably before they metastasize, show overt symptoms, or cause serious organ damage) has long been an unmet goal of medicine. Much of the difficulty in routine early detection of tumors has been in identifying some property which is common to most or all tumors, and which can be effectively exploited for routine and affordable early detection of tumors. An onco-tool carrying a suitable radioisotope (e.g., a gamma or positron emitter) which emits a signal readily detectable outside the body, combined with modern imaging technologies, is designed to safely provide very early detection of most or all tumor types.

A diagnostic onco-tool is designed to be broadly used in routine annual physical exams to provide very early detection of essentially all tumors larger than microscopic size. Accordingly, a single diagnostic onco-tool may serve as a superior and comprehensive replacement for a wide range of current tumor diagnostic procedures, such as breast mammograms, pap smears, prostate exams, and colonoscopies. Furthermore, it should be appreciated that many tumor types currently cannot be routinely detected until they reach a quite late stage of development wherein they manifest themselves by pain, malfunction of an organ, or some other generic symptom or set of symptoms, which then require exhaustive and very expensive diagnostic procedures in order to identify the root cause of the problem. By the time such a tumor is detected it is often too advanced for any hope of successful treatment (at least with currently approved therapies). In this regard, diagnostic onco-tools offer the promise of an entirely new capability for routine early-stage detection of such difficult-to-diagnose tumors.

Therapeutic onco-tools:

Therapeutic onco-tools are designed to exploit the acidity characteristic of tumors to provide safe and effective destruction of tumors. For optimal results a combination of two onco-tool formulations should be used. One formulation

carries a radioisotope which emits short-path-length high linear energy transfer radiation, such as a very-high-energy alpha particle, that is effective to kill the close-by radiation-resistant quiescent cells in acidic areas of the tumor (see Figure 2). The other formulation carries a radioisotope that emits a medium-path-length medium-energy beta particle effective for killing the more-distant radiation-sensitive fast-dividing tumor cells near capillaries. When used in combination, these two formulations are designed to effectively kill the entire tumor, with little or no damage to other cells in the body. As a consequence, therapeutic onco-tools are designed to be effective against all cell types in most or all tumors, and so offer the promise of far less toxic and far more effective treatment of tumors, with few or no relapses.

DETAILED DESCRIPTION OF THE INVENTION:

BRIEF DESCRIPTION OF THE FIGURES:

Figure 1 shows the weak-acid cytotoxic chemotherapeutic, chlorambucil, in both its anionic and its non-ionic forms.

Figure 2 illustrates the distribution of acidity in tumors.

Figure 3 illustrates the pH-mediated and solubility-mediated transitions between forms.

Figure 4 shows calculated titration curves as a function of lipophilicity of the non-ionic acid form.

Figure 5a illustrates expected waters of hydration, showing water molecules H-bonded to a conventional carboxylic acid in its anionic and non-ionic forms.

Figure 5b illustrates expected waters of hydration, showing water molecules H-bonded to a carboxylic acid-containing structure in its anionic form and in its internally H-bonded non-ionic form.

Figure 6a illustrates an acid-specific H-bond in a structure which forms an internal acid-specific H-bond only in its non-ionic form.

Figure 6b illustrates a structure which forms non-acid-specific H-bonds in both its anionic form and in its non-ionic form.

Figure 7a shows representative ring structures suitable for advanced pH-switches, including a 4-membered ring structure, a 5-membered ring structure, and a 6-membered ring structure.

Figure 7b shows a structure which is unacceptable for use as an advanced pH-switch because of it has an acyclic structure.

Figure 8a illustrates the insulation of carboxyl moieties from inductive effects due to an increasing number of carbons separating the carboxyl from an electron-withdrawing group.

Figure 8b illustrates a structure having inadequate insulation of its carboxyl from inductive effects.

Figure 9a illustrates a structure whose H-bond site is open to solvent.

Figure 9b illustrates a structure whose H-bond site which is partially shielded from solvent.

Figure 10 shows components of low-barrier H-bonds.

Figure 10a shows an H-bond donor, comprising a carboxylic acid, which is suitable for forming low-barrier H-bonds with H-bond acceptor moieties having a pKa value, in the stand alone form, in the range of approximately 3.0 to 6.5.

Figure 10b shows representative H-bond acceptor moieties suitable for forming low-barrier H-bonds with a carboxylic acid donor.

Figure 10c illustrates representative advanced pH-switches designed to form internal acid-specific low-barrier H-bonds.

Figure 11 illustrates the statistical basis for increased specificity of multi-pH-switch onco-tools.

Figure 11a illustrates non-sequestered anionic and tumor-sequestered non-ionic forms of an onco-tool with two pH-switches.

Figure 11b illustrates non-sequestered anionic and tumor-sequestered non-ionic forms of an onco-tool with three pH-switches.

Figure 12 shows calculated efficacy and specificity factors for multi-pH-switch onco-tools.

Figure 12a shows calculated efficacy and specificity factors as a function of pKa value for structures containing one pH-switch.

Figure 12b shows calculated efficacy and specificity factors as a function of pKa value for onco-tools containing two pH-switches.

Figure 12c shows calculated efficacy and specificity factors as a function

of pKa value for onco-tools containing three pH-switches.

Figure 12d shows calculated efficacy and specificity factors as a function of pKa value for onco-tools containing four pH-switches.

Figure 13 shows a composition containing two advanced pH-switches.

Figure 13a shows the structure of this composition.

Figure 13b shows a plot of the n-Octanol/buffer partitioning of this 2-pH-switch composition as a function of pH.

Figure 14 illustrates representative cargo components in both their precursor and final forms.

Figure 15 illustrates two synthetic routes for representative cargo components, including a simple procedure for converting selected precursor forms to their final forms.

Figure 16 illustrates representative 2-pH-switch onco-tools.

Figure 16a illustrates two representative 2-pH-switch onco-tools containing simple pH-switches.

Figure 16b illustrates five representative 2-pH-switch onco-tools containing advanced pH-switches.

Figure 16c illustrates ten representative 2-pH-switch onco-tools containing advanced pH-switches designed to form low-barrier H-bonds. Structures 1 and 2 of Figure 16c show two onco-tools wherein the H-bond acceptor moiety of the pH-switches are N-oxide moieties, and wherein each N-oxide moiety serves as the H-bond acceptor for two carboxylic acid H-bond donor moieties.

Figure 17 illustrates representative 3-pH-switch onco-tools.

Figure 17a illustrates two representative 3-pH-switch onco-tools containing advanced pH-switches.

Figure 17b illustrates two representative 3-pH-switch onco-tools containing advanced pH-switches designed to form low-barrier H-bonds.

Figure 18 illustrates representative 4-pH-switch onco-tools.

Figure 18a illustrates a 4-pH-switch onco-tool where the H-bond acceptor moieties of the pH-switches are alkoxy amine moieties.

Figure 18b illustrates a 4-pH-switch onco-tool where the H-bond acceptor moieties of the pH-switches are N-oxide moieties, and wherein each N-oxide moiety serves as the H-bond acceptor for two carboxylic acid H-bond donor moieties.

Figure 19 illustrates synthesis of onco-tools containing simple pH-switches, wherein lipophilicity can be adjusted by varying the R group.

Figure 20 illustrates a synthetic scheme for advanced pH-switches, wherein lipophilicity can be adjusted by varying the R group.

Figure 21 illustrates useful amine-ester and ketone-ester intermediates for onco-tool synthesis.

Figure 22 illustrates representative syntheses of pH-switches designed to form an internal acid-specific low-barrier H-bond.

Figure 22a shows the synthesis of a pH-switch wherein the H-bond acceptor is a cyanomethyl amine moiety.

Figure 22b shows the synthesis of a pH-switch wherein the H-bond acceptor is an N-oxide moiety.

Figure 22c shows the synthesis of a pH-switch wherein the H-bond acceptor is a trifluoroethyl amine moiety.

Figure 23 shows titration results for a variety of pH-switch structures.

Figure 23a shows a conventional titration curve, where the titration was carried out in Methanol/Water, 1:1 by vol., for a simple carboxylic acid (Butyric acid) and an advanced pH-switch (an acid-amide derivative of Camphoric acid), plus that same data plotted in the more informative first derivative form.

Figure 23b shows a conventional titration curve, where the titration was carried out in water and each specie was present at 33 milliMolar concentration, for two-pH-switch onco-tools (but with stable Iodine instead of radioactive Iodine) varying in their R group, and a related three-pH-switch structure.

Figure 23c shows a titration curve, plotted as the first derivative, for an advanced pH-switch designed to form a low-barrier H-bond. This titration was carried out in water and the pH-switch, comprising an N-oxide/acid structure derived from Camphoric acid, was present at a 5 milliMolar concentration.

Figure 24 shows experimentally-determined pKa values for three pH-switch structures, including: the amide/acid advanced pH-switch derived from Camphoric acid, shown in Figure 23a; an N-oxide/acid advanced pH-switch designed to form a low-barrier H-bond, derived from Isonipocotic acid; and, the N-oxide/acid advanced pH-switch designed to form a low-barrier H-bond, derived from Camphoric acid, shown in Figure 23c.

Figure 25 illustrates synthetic schemes for representative onco-tools containing advanced pH-switches.

Figure 25a shows an onco-tool wherein its two pH-switches are joined by a di-acylhydrazide structure.

Figure 25b shows an onco-tool wherein its two pH-switches are joined by a di-amide structure.

Figure 26 illustrates synthetic schemes for representative onco-tools containing advanced pH-switches designed to form a low-barrier H-bond.

Figure 26a shows a synthetic scheme for a 2-pH-switch onco-tool wherein a single N-oxide moiety serves as the H-bond receptor moiety for two carboxylic acid H-bond donor moieties.

Figure 26b shows a synthetic scheme for a 2-pH-switch onco-tool wherein both of the nitrogens of a hydrazine moiety serve as the H-bond receptor moieties for two carboxylic acid H-bond donor moieties.

Figure 26c shows a synthetic scheme for a 2-pH-switch onco-tool wherein cyanomethyl amine moieties serve as the H-bond acceptor moieties for the carboxylic acid H-bond donor moieties.

Figure 26d shows a synthetic scheme for a 4-pH-switch onco-tool wherein each N-oxide moiety serves as the H-bond receptor moiety for two carboxylic acid H-bond donor moieties.

DEFINITIONS OF TERMS:

The terms used herein have the following specific meanings, unless otherwise noted.

The words "comprising," "having," "containing," and "including," and other forms thereof, are intended to be equivalent in meaning and be open ended in that an item or items following any one of these words is not meant to be an exhaustive listing of such item or items, or meant to be limited to only the listed item or items.

The words "consisting," "consisting of," and other forms thereof, are intended to be equivalent in meaning and be closed ended in that an item or items following

any one of these words is meant to be an exhaustive listing of such item or items and limited to only the listed item or items.

pH-switch component – a structural component of an onco-tool which is capable of undergoing a pH-mediated transition between an anionic hydrophilic form at a higher pH and a non-ionic lipophilic form at a lower pH.

Advanced pH-switch component – a pH-switch component designed to form an internal acid-specific hydrogen bond. An advanced pH-switch has the following properties:

a) contains an aliphatic ring structure selected from the group consisting of: 4-membered rings, 5-membered rings, and 6-membered rings;

b) contains a carboxylic acid moiety directly linked to the aliphatic ring structure;

c) the carboxylic acid moiety is separated from any linked electron-withdrawing group by at least two carbons;

d) contains an H-bond acceptor moiety selected from the group consisting of:

i) part of the aliphatic ring structure;

ii) directly linked to the aliphatic ring structure; and

iii) linked through one atom to the aliphatic ring structure;

e) the H-bond acceptor moiety has a structure which in its non-ionic form does not serve as an H-bond donor moiety; and

f) the carboxylic acid moiety and said H-bond acceptor moiety are positioned in close proximity and are properly positioned and oriented such that they are compatible with formation of an internal acid-specific H-bond.

Cargo component – a structural component of an onco-tool which serves to bind a radioisotope that is effective to report the presence of the onco-tool, or is effective to kill cells. The cargo component can exist in either a precursor form ready to bind a radioisotope or a final form which contains a radioisotope.

Cargo component in precursor form – a cargo component which has a structure that is capable of readily binding to a selected radioisotope, but has not yet bound a radioisotope. An onco-tool with this precursor form of the cargo

component is suitable for long-term storage.

Cargo component in final form – a cargo component that contains at least one bound radioisotope. An onco-tool with this final form of the cargo component is suitable for delivery into a subject for the purpose of detecting and/or killing tumors containing acidic areas.

Onco-tool – a non-peptide composition that includes at least two pH-switch components and at least one cargo component..

Diagnostic onco-tool – an onco-tool which contains a radioisotope that is effective to report the presence of the onco-tool within a tumor to a detector outside the subject undergoing the diagnostic procedure.

Therapeutic onco-tool – an onco-tool which contains a radioisotope that is effective to kill cells.

Dual-radioisotope onco-tool therapy strategy – a treatment strategy where at least two different onco-tools are used to treat tumors, where one onco-tool contains a radioisotope which emits high linear transfer energy radiation, and another onco-tool contains a beta-emitting radioisotope.

Interstitial space – the area of a tissue or of a tumor which is outside of the vascular bed and outside of the cells.

Acidic area of tumor – an area of a tumor where the interstitial space has a pH of less than 7.0.

Physiological conditions – an aqueous solution with a temperature in the range of 20 deg. C to 40 deg. C and having a sodium chloride concentration between about 0.13 M and 0.17 M.

Significant portion – greater than approximately 1%.

Anionic hydrophilic form – a form which carries at least one negative charge and has an octanol/water partitioning coefficient of less than 1.

Non-ionic lipophilic form – a form which does not carry an ionic charge and has an octanol/water partitioning coefficient of greater than 1.

Efficacy factor – the percent of the onco-tool molecules which are in their non-ionic form at pH 6.4.

Specificity factor – the ratio: (percent of onco-tool molecules in non-ionic form at pH 6.4) divided by (percent of onco-tool molecules in non-ionic form at pH 7.4).

Effective to report the presence – a radioisotope whose emission, such as a gamma ray or positron, generates a signal which is readily detectable outside that subject.

pT – the pH value at the mid-point of the transition between the hydrophilic form of a substance and the lipophilic form of that substance, where that transition between the two forms is measured with the substance present at 5 milliMolar in aqueous physiological saline (or a close equivalent containing some buffer). While the pT value resembles the pKa value for a substance, the pT value differs in that it is influenced both by the pKa of the substance and the lipophilicity of the substance. The pT value is useful for describing carboxyl-containing substances whose non-ionic form is strongly lipophilic and so can drop out of aqueous solution during titrations, or can partition into a non-polar phase during partitioning assays. Further, the pT value is measured in the presence of approximately a physiological concentration of saline so it better reflects properties of the test substance in an in vivo environment.

Radioisotope which emits high linear energy transfer radiation – a radioisotope which emits an alpha particle or which emits an Augar and/or Coster-Kronig electron.

Inventor – James E. Summerton, Ph.D.

OUTLINE OF THE DISCLOSURE:

A. Molecular Design of Onco-Tools
    1. pH-Switch Components
        a) Adjust lipophilicity of pH-switch
        b) Incorporate a novel acid-specific internal H-bond in pH-switch
        c) Incorporate multiple pH-switch components in onco-tool
    2. Cargo Component
        a) Structural requirements
        b) Precursor and final forms
        c) Selection of radioisotope cargo
            i) for detecting tumors
            ii) for treating tumors
    3. Onco-Tool Structures
        a) Structural requirements
        b) Onco-tools with 2 pH-switches
        c) Onco-tools with 3 pH-switches
        d) Onco-tools with 4 pH-switches

B. Synthesis, Testing, and Optimization of Components and Onco-Tools
    1. Preparation of structures containing representative pH-switches
        a) Simple pH-switches
        b) Advanced pH-switches
        c) Advanced pH-switches designed to form a low-barrier H-bond
    2. Testing pH-switches
    3. Preparation of representative onco-tools and conversion to final form
        a) Onco-tools with simple pH-switches
        b) Onco-tools with advanced pH-switches
        c) Onco-tools with advanced pH-switches designed to form a low-barrier H-bond

4. Testing and optimization of onco-tools
    a) Iterative optimization process: synthesis and testing in cultured cells
        i) Prepare set of onco-tools varying in lipophilicity
        ii) Assess entry into cultured cells at pH 6.4 and pH 7.4
        iii) Repeat above steps as needed
    b) Assess disposition in normal mice
    c) Assess disposition in tumor-bearing mice
    d) Preclinical and clinical testing

C. Methods of Using Onco-Tools
1. Diagnostic method
2. Therapeutic method
    a) Single-radioisotope method
    b) Dual-radioisotope method
3. Comprehensive method for detecting and treating tumors
4. Strategy for dealing with micro-metasteses
5. Treat to decrease pH in tumors for increased efficacy and specificity
6. Use multiple onco-tools varying in pKa to increase efficacy and specificity
7. Treat to increase pH in urine to protect kidneys from damage
8. Monitor and flush bladder for increased safety

MODES FOR CARRYING OUT THE INVENTION:

A. Molecular Design Of Onco-Tools

Onco-tools comprising the present invention are a novel class of molecules designed for detection and treatment of tumors. Each onco-tool contains two or more pH-switch components which readily undergo a pH-mediated transition between an anionic hydrophilic form at higher pH and a non-ionic lipophilic form at lower pH. Each onco-tool also contains a cargo

component which is effective to bind a selected radioisotope that is suitable for carrying out the diagnostic or therapeutic role of the onco-tool.

1. pH-Switch Components

If an onco-tool is to achieve adequate specificity for acidic areas of tumors it must have a structure such that in aqueous solution at pH 7.4 nearly all of the onco-tool molecules exist in a negatively-charged form (anionic) which lacks affinity for the negatively-charged surfaces of cell membranes. Conversely, in order to achieve adequate efficacy, at the pH present in acidic areas of tumors a significant portion (greater than 1%) of the onco-tool molecules should switch to a non-ionic lipophilic form that readily enters cell membranes. A principal challenge in designing onco-tools is to devise a structure wherein a sufficient portion of the onco-tool molecules undergo the transition between the anionic hydrophilic form and the non-ionic lipophilic form within the available very limited pH difference between normal tissues and acidic areas of tumors. While this pH difference is commonly only about 0.5 to 0.7 pH unit, it should be appreciated that simple steps (described in Section C later herein) can be easily implemented to substantially increase this pH difference between normal tissues and acidic areas of tumors. With such steps the pH difference between normal and acidic areas of tumors can typically be increased to 1.0 pH unit or greater.

To meet the very demanding molecular design challenge inherent in exploiting the acidity in tumors, pH-switch structures have been devised wherein each contains a weak acid moiety (generally a carboxylic acid) that carries a negative charge at pH 7.4. Then, because this essential weak-acid moiety still exhibits substantial hydrophilic character in its non-ionic free-acid form, in order to achieve adequate efficacy two molecular design strategies have been utilized in order to provide sufficient lipophilicity for rapid membrane entry when the onco-tool enters an acidic area in a tumor . These two design strategies, used either singly or in combination, when combined with a third design strategy, can also play a key role in dramatically increasing the onco-tool's specificity for acidic areas of tumors. The physical and mathematical basis for these three molecular design strategies, two of which are believed to be unique to onco-tools, are described below.

a) Adjust lipophilicity of pH-switch by incorporating multiple lipophilic moieties to partially counter the inherent hydrophilicity of the free-acid moiety. In this regard it is first useful to obtain a quantitative assessment of how increasing lipophilicity of the non-ionic free-acid form of the pH-switch affects its partitioning properties (ie., how it partitions between an aqueous phase and a lipophilic phase such as octanol or a cell membrane). To this end, mathematical modeling was carried out to calculate the pH-dependent transition between forms of a weak-acid substance which exists at high pH in an anionic water-soluble form, [A-], and which converts at a lower pH to a non-ionic lipophilic form which has some aqueous solubility, [HAsol], and which can also become water-insoluble/lipid soluble, denoted by [HAinsol], by virtue of precipitating or oiling out of aqueous solution, or by virtue of partitioning into a lipophilic phase such as octanol or a cell membrane. The inter-conversions of these three forms, along with the key equations used in the mathematical modeling, are shown in Figure 3.

The results from that modeling, shown in Figure 4, demonstrate that the pH of the mid-point of the transition between the anionic form, [A-], and the non-ionic forms, [HAsol plus HAinsol], progressively rises as the octanol/water partitioning coefficient, P, of the non-ionic free-acid form is increased. However, such an increase in the pH of the transition will only apply after the free-acid form reaches the lipophilicity threshold effective to initiate precipitation or oiling out, or if a non-polar phase is present to allow partitioning. Example 1 describes titration experiments which illustrate this mathematically-predicted impact of the lipophilicity of the acid form on the pH-dependent transition between the anionic and non-ionic forms.

However, it should be noted that incorporating excessive lipophilic moieties as the sole means for increasing the lipophilicity of the non-ionic form can be counter productive. This is because when a very high lipophilicity is generated solely by incorporation of an excessive number of lipophilic moieties then even the anionic form of the pH-switch can begin to have an affinity for cell surfaces - resulting in sequestering of the onco-tool in normal tissues as well as in tumors. This can be a particular problem when the lipophilic moieties form one large surface and the hydrophilic moieties are well separated from this lipophilic surface. When the onco-tool is also sequestered in normal tissues it will cause

high background signal in the diagnostic application, and significant toxicity in the therapeutic application.

b) Incorporate a novel acid-specific internal H-bond in pH-switch to amplify the solubility differential between the hydrophilic and lipophilic forms.

In the foregoing section a rather conventional strategy was described for improving cell entry of onco-tool into cells in acidic areas of tumors, and thereby increasing efficacy, that strategy being to incorporate lipophilic groups into the pH-switch components. This simple strategy is commonly used in what is referred to as "rational drug design" - which has been widely applied in attempts to improve the efficacy and specificity of cancer chemotherapeutics. However, because there is only a rather small difference in pH values between normal tissues and acidic areas of tumors, the inventor set out to devise an additional strategy for enhancing entry of onco-tools into acidic areas, which it was hoped could also increase the onco-tool's specificity for tumors. To this end, the inventor postulated that both the hydrophilicity of the anionic form and the lipophilicity of the non-ionic form of the pH-switches could be substantially increased by devising a structure effective to form a pH-dependent internal acid-specific hydrogen bond. The inventor further postulated that such an internal H-bond would raise the pH at which the pH-switch transitioned between its anionic form and its non-ionic form, thereby making more of the onco-tool available in the non-ionic form for entry into cells in acidic areas of tumors.

That predicted H-bond-mediated increase in lipophilicity of the non-ionic form and concomitant increase in hydrophilicity of the anionic form is based on the expectation that in an aqueous environment such an internal H-bond will displace several (probably two) bound waters from the free-acid form. To illustrate, Figure 5a shows the expected waters of hydration directly H-bonded to a standard carboxyl in its anionic form and in its non-ionic free-acid form. Figure 5b shows corresponding expected waters of hydration directly H-bonded to the anionic form and to the non-ionic free-acid form of a structure effective to form the desired internal acid-specific H-bond. Clearly, both the standard carboxyl and the carboxyl effective to form an internal H-bond lose their counter-ion in going from the anionic form to the non-ionic free-acid form. However, while the standard carboxyl is expected to have the same number (probably 4) of waters

directly H-bonded to both the anionic form and to the non-ionic free-acid form, the inventor believes that for a carboxyl able to form an internal H-bond there will be a net loss of several (probably two) directly-H-bonded waters when going from the anionic form (probably 5 H-bonded waters) to the non-ionic free-acid form (probably 3 H-bonded waters) - and this loss of several H-bonded waters will substantially amplify the hydrophilicity/lipophilicity differential between the two forms. The inventor predicted that if such an internal acid-specific H-bond could be formed in an aqueous environment, then the resulting loss of several waters of hydration would significantly increase an onco-tool's ability to rapidly enter cells in acidic areas of tumors, while also significantly increasing the onco-tool's repulsion from cells in normal tissues. In summary, incorporation of such an internal acid-specific H-bond was predicted to increase both the specificity and the efficacy of an onco-tool by differentially altering the number of water molecules bound to each of the two forms of its component pH-switches.

The inventor also predicted (and has now demonstrated) that formation of an internal acid-specific H-bond in the pH-switch component should favor the transition of the carboxyl moiety of a pH-switch component to its free-acid form, resulting in a significant increase in the pKa value for that carboxylic acid moiety. As will be detailed in the next section, such an increase in the pKa of the carboxyl moiety is desirable in the context of a strategy for dramatically increasing the onco-tool's specificity for tumors by incorporating two or more pH-switches in that onco-tool.

In regard to designing pH-switch structures capable of forming an internal H-bond in an aqueous environment, conventional wisdom among experts in hydrogen bonding typically holds that a lone hydrogen bond will not be stable in an aqueous environment because of competition with water's vast concentration of H-bond acceptor sites (110 Molar) and H-bond donor sites (110 Molar). Instead, it is commonly believed that a stable non-covalent interaction in aqueous solution requires a multiplicity of interactions, selected from H-bonds, hydrophobic interactions, and electrostatic interactions.

While this requirement for a multiplicity of non-covalent bonds appears to be well established for inter-molecular interactions, the inventor postulated that, contrary to the conventional wisdom, it might be possible to devise compact structures which will form a single relatively stable pH-dependent intra-molecular

H-bond in aqueous solution, where that H-bond both increases the hydrophilicity/lipophilicity differential between the two forms, and serves to significantly favor the free-acid form over the anionic form (ie., raise the pKa). The crucial question then was: could practical pH-switch structures be devised which would form such an internal H-bond in aqueous solution?

After considerable experimentation, novel structures have now been devised which have been demonstrated to form the desired single pH-dependent internal H-bond, and which are suitable for incorporation into onco-tools of the present invention. As initially predicted by the inventor, such an internal H-bond appears to significantly enhance the hydrophilicity/lipophilicity differential when the pH-switch goes from its anionic form to its non-ionic form. Several such internal acid-specific H-bonds have also been demonstrated to significantly increase the pH at which the structure switches from its anionic hydrophilic form to its non-ionic lipophilic form, relative to the pH at which a similar simple carboxyl moiety undergoes this transition.

A pH-Switch which has a structure designed to form an internal acid-specific H-bond is called an "advanced pH-switch". Results from molecular modeling and from extensive experimental work suggest that the following three properties are essential in order for an advanced pH-switch to form an acceptably stable internal acid-specific H-bond in aqueous solution.

i) Acid-specific H-bond

The structure must contain a carboxyl moiety which is positioned in suitable proximity to an H-bond acceptor moiety for formation of an H-bond. When that carboxyl moiety is in its free-acid form it must serve as the H-bond donor, and the proximal H-bond acceptor moiety must be such that in its non-ionic form it can only serve as an H-bond acceptor, and cannot serve as an H-bond donor. The inventor refers to an H-bond formed by such a structure as an "internal acid-specific H-bond". Figure 6a shows a representative pH-switch structure which can form only an internal acid-specific H-bond. Conversely, Figure 6b illustrates a similar, but unacceptable structure which can form both an internal H-bond when the carboxyl is in its anionic form and an internal H-bond when the carboxyl is in its free-acid form. The inventor calls this dual H-bonding capability "internal non-acid-specific H-bonding," and his experimental results

indicate that such non-acid-specific H-bonding is unacceptable because it fails to provide the desired increase in the lipophilicity of the acid form, and it fails to raise the pH at which the structure switches from its anionic hydrophilic form to its non-ionic lipophilic form. Example 2 describes experimental results wherein in a 1 to 1 mixture of water and methanol the structure in Figure 6a forms an internal acid-specific H-bond under mildly-acidic conditions - evidenced by a significant increase in the pH of the transition between anionic and free-acid forms (that is, an increase in the pKa value for the carboxyl moiety). Conversely, this same example also shows that the very similar structure in Figure 6b, designed to form non-acid-specific H-bonds, fails to increase the pH of the transition between anionic and non-ionic forms under these conditions.

ii) Minimal conformation freedom between H-bonding moieties

The H-bond acceptor moiety and the carboxyl serving as the H-bond donor moiety should be held in close proximity to each other by a structure which has minimal conformational freedom. This limited conformational freedom can be achieved by using a suitable ring structure. Molecular modeling and experimental work suggests that 4-membered, 5-membered, and 6-membered aliphatic rings are preferred for this purpose. Figure 7a shows representative 4-membered, 5-membered and 6-membered aliphatic ring structures which allow only limited conformational freedom between the H-bond donor (OH of the carboxylic acid) and suitably-positioned H-bond acceptor moieties. Conversely, Figure 7b shows a somewhat similar structure which is unacceptable because its acyclic structure allows excessive conformational freedom between the H-bond donor and H-bond acceptor moieties. Example 3 describes experimental results showing that in a 1:1 mix of methanol and water a structure with minimal conformational freedom between H-bonding moieties can afford a significant increase in the pKa value of the carboxyl moiety, while a similar structure with substantially greater rotational freedom between H-bonding moieties fails to provide a corresponding increase in the pKa value of the carboxyl moiety - and so is presumably failing to form a stable internal acid-specific H-bond under these conditions.

iii) Carboxyl insulated from inductive effects of electron-withdrawing groups

The carboxylic acid which is to serve as the H-bond donor moiety should be separated from any linked electron-withdrawing group by at least two, and preferably three or more carbons. This avoids any excessive reduction in the pKa value of that carboxyl due to inductive effects from electron-withdrawing groups. The molecular structures and their corresponding pKa values shown in Figure 8a illustrate how an increasing number of aliphatic carbons can progressively insulate a carboxyl from the pKa-reducing effects of a phenyl group. Figure 8b shows a structure wherein inductive effects from the proximal amide moiety apparently causes an undue reduction in the pKa of the carboxylic acid moiety. Example 4 describes experimental results suggesting that even with minimal conformational freedom between H-bond donor and acceptor moieties, if there is inadequate insulation of the carboxyl from inductive effects then the pH of the transition between anionic and free-acid forms will be too low to be useful in a pH-switch.

In addition to the above 3 essential properties, it appears that at least one additional property, selected from the following two properties, is desirable to achieve formation of an internal H-bond in aqueous solution.

iv) Lipophilic groups partially shielding H-bonding site

A principal challenge in forming a lone H-bond in an aqueous environment is to preferentially form that H-bond in the presence of the vast concentration of competing H-bond donors and H-bond acceptors comprising the surrounding water. Based both on biochemical studies of enzyme catalytic sites and on the inventor's extensive molecular modeling, the inventor postulated that the desired intra-molecular H-bond might be more favored if the H-bonding site were partially shielded from the bulk water by the presence of lipophilic groups. In the context of partial shielding from the solvent, Figure 9 shows two relevant structures. In Figure 9a the H-bonding site is quite open to the solvent. Conversely, in Figure 9b the corresponding H-bonding site is partially shielded from the solvent by an adjacent methyl on one side and by an adjacent methylene on the other side. Syntheses and titration studies, detailed in Example 5, give results which suggest such partial shielding of the H-bonding site from the solvent favors the desired

intramolecular H-bond.

v) Low-barrier H-bond

The term "low-barrier H-bond" is used herein to mean a non-covalent bond formed between an H-bond donor moiety and an H-bond acceptor moiety, where the pKa values of the two isolated moieties are within about 2 pH units of each other. It should be noted that this definition includes what can also be construed as an internal salt wherein the hydrogen is closer to the acceptor moiety than to the donor moiety. Such low-barrier H-bonds are commonly found to be exceptionally strong and so can appreciably favor the desired intramolecular H-bond in pH-switches. Figures 10a and 10b show a variety of H-bonding moieties which are appropriate for forming low-barrier H-bonds in pH-switches. Figure 10c illustrates several representative pH-switch structures designed to form H-bonds of the low-barrier type. Example 6 describes the syntheses and testing of two representative pH-switches (structures i and iii in Figure 10b), along with experimental evidence for formation of the desired low-barrier H-bonds in aqueous solution.

Below is a summation of properties which have been found to be suitable for an advanced pH-switch component which is designed to form an internal acid-specific hydrogen bond:

a) contains an aliphatic ring structure selected from the group consisting of: 4-membered rings, 5-membered rings, and 6-membered rings;

b) contains a carboxylic acid moiety directly linked to the aliphatic ring structure;

c) the carboxylic acid moiety is separated from any linked electron-withdrawing group by at least two carbons;

d) contains an H-bond acceptor moiety selected from the group consisting of:

i) part of the aliphatic ring structure;

ii) directly linked to the aliphatic ring structure; and

iii) linked through one atom to the aliphatic ring structure;

e) the H-bond acceptor moiety has a structure which in its non-ionic form does not serve as an H-bond donor moiety; and

f) the carboxylic acid moiety and the H-bond acceptor moiety are positioned in close proximity to each other and are properly positioned and oriented

such that they are compatible with formation of an internal acid-specific H-bond.

c) Incorporate multiple pH-switch components into each onco-tool as a novel strategy for dramatically increasing the onco-tool's specificity for tumors - without causing an undue reduction in the onco-tool's efficacy.

The two design strategies described in the previous two sections, used singly or in combination, serve to generate pH-switch structures which are effective to enter cell membranes at the pH present in acidic areas of tumors, and so provide good efficacy. However, such design strategies can provide only a modest specificity advantage over conventional cancer therapies, such as chlorambucil. In this regard, it should be appreciated that with most currently-used cancer therapies it is their poor specificity which generally underlies their many failures to cure patients. That is, current cancer therapies are estimated to have specificities on the order of only about 2 to 8. This means they are typically on the order of 2 to 8 fold more damaging to cancers than to normal tissues. The inventor was acutely aware that a similar limited specificity level and corresponding low success rate would likely plague onco-tool therapeutics unless some effective strategy could be devised to substantially increase their specificity for tumors. Accordingly, several years ago the inventor embarked on a quest to devise some way to substantially increase the specificity of onco-tools. That quest led to the third and most essential onco-tool design strategy, described below.

The pKa of an acid is the pH of an aqueous solution of that acid wherein half of the acid molecules are anionic and half are non-ionic. If one knows the pKa of an acid, and the pH of its aqueous solution, then the Henderson-Hasselbalch equation can be used to calculate the ratio of anionic, [H-], to non-ionic, [HA], forms of that acid present in that solution: ($pH = pKa + \log [A^-] / [HA]$).

A simple application of the Henderson-Hasselbalch equation suggests that if the pH in the interstitial space in normal tissues and the pH in acidic areas of tumors differ by only 1.0 pH unit (ie., about pH 6.4 achievable in tumors and pH 7.4 present in normal tissues) then the theoretical maximum specificity a carboxyl-containing drug could show for acidic areas of a tumor, relative to normal tissues, appears to be no more than 10. Stated differently, with only a 1.0

pH differential between normal and tumor a simple application of the Henderson-Hasselbalch equation appears to suggest that the rate of entry of a conventional weak-acid-containing drug into a cell in an acidic area of a tumor can be no more than about 10 fold higher than the rate of entry of that drug into a cell in a normal tissue.

If this upper limit of about 10 were to apply to the specificity of onco-tools, then it is likely that in the diagnostic application this poor specificity level would generate such a large background signal from normal tissues that it might swamp out much or all of the signal from any tumors one was attempting to detect - particularly for the case of very small early-stage tumors whose detection would provide the best prognosis for the patient. Likewise, in a therapeutic application such a poor specificity level would likely cause considerable toxicity in the patient, possibly to the extent of killing the patient - which commonly occurs with current chemotherapeutics which also have similar poor specificity levels.

To overcome the problem of limited specificity, the inventor has devised a novel design strategy to dramatically increase the specificity of onco-tools over the apparent theoretical maximum of 10 which might appear to be imposed by the small pH differential between normal and tumor tissues. In essence, the strategy is to incorporate two or more pH-switches into a single onco-tool, where the carboxyl of each pH-switch is sufficiently distant (probably greater than about 5 angstroms apart) from the carboxyl of its neighboring pH-switch that ionization of one carboxyl does not significantly affect the ionization of its neighboring carboxyl. Conversely, all of the pH-switches of the multi-pH-switch onco-tool should be in sufficiently close proximity within the onco-tool molecule that all of the component pH-switches must be in their non-ionic form before that onco-tool can closely approach and then breach the negatively-charged outer surface of a cell membrane in order to enter its lipophilic interior, as is required for that onco-tool to be sequestered in acidic areas of tumors. It is estimated that all carboxyls of a multi-pH-switch onco-tool should be no more than approximately 15 to 20 angstroms from each other in order to adequately satisfy this latter requirement.

Figure 11a illustrates in an abstract form the various anionic and non-ionic forms of an onco-tool containing two pH-switch components, and Figure 11b illustrates likewise the various anionic and non-ionic forms of an onco-tool containing three pH-switch components. It should be appreciated that it is the

concentration of the non-ionic form which is of particular interest because, when properly designed, it should be only this non-ionic form of the onco-tool which is capable of efficiently contacting and entering cell membranes - with all the other forms containing at least one anionic moiety being repelled from the anionic cell surfaces by electrostatic forces.

When employing this multi-pH-switch design strategy one can calculate the expected efficacy and specificity factors for a given onco-tool using the pKa value for the carboxyl moiety of the pH-switch used in that onco-tool, and the number of those pH-switches in that onco-tool. In these calculations, the efficacy factor is the percent of the onco-tool molecules which are in their non-ionic form at pH 6.4. This factor provides a measure relating to how efficiently the onco-tool will enter cells in acidic areas of tumors. The specificity factor is the ratio: (percent in non-ionic form at pH 6.4) divided by (percent in non-ionic form at pH 7.4). This factor provides a measure relating to the relative rate of entry of the onco-tool into cells in acidic areas of tumors (at pH 6.4) relative to the rate of entry of the onco-tool into cells in normal tissues (at pH 7.4). For these calculations one first uses the Henderson-Hasselbalch equation to calculate for the selected pH-switch what percent will be in the non-ionic form at pH 6.4 (achievable in acidic areas of tumors) and at pH 7.4 (present in normal tissues). Then a binomial expansion is used to calculate the percent of onco-tool molecules which have all of their component pH-switches in the non-ionic form.

Figure 12 shows plots of calculated efficacy and specificity factors for a) compositions with 1 pH-switch, b) onco-tools with 2 pH-switches, c) onco-tools with 3 pH-switches, and d) onco-tools with 4 pH-switches - as a function of the pKa values of their component pH-switches. These calculated efficacy and specificity factors demonstrate that compositions containing a single carboxyl moiety, such as chlorambucil, inherently have rather poor specificities for selective entry into cells in acidic areas of tumors (specificity factor is always less than 10). However by incorporating two or more pH-switches in a single onco-tool, the calculations suggest that with practical efficacy factors (over about 5% of onco-tool in non-ionic form at pH 6.4) and with proper adjustment of the pKa value (see earlier section on internal acid-specific H-bonds), the specificity factors of onco-tools can be increased many fold over that possible for single-carboxyl compositions. In particular, with suitable pKa values the specificity

factor can be over 50 for onco-tools containing 2 pH-switches, and over 200 for onco-tools containing 3 pH-switches, and over 900 for onco-tools containing 4 pH-switches.

As can be seen from Figure 12, there is an inverse relationship between the efficacy factor and the specificity factor. Thus, with increasing pKa of the pH-switch the efficacy factor increases while the specificity factor decreases. Since reasonable efficacy and high specificity are both desired in a cancer therapeutic, it is useful to consider what pKa value will likely provide a reasonably optimal balance between efficacy and specificity. Based on the calculated values in Figure 12b it appears that a pH-switch with a pKa of 5.9 should provide a desirable balance between efficacy (6%) and specificity (60) for onco-tools containing 2 pH-switches. Further, calculated values in Figure 12c suggest that a pH-switch with a pKa of 6.2 should provide a desirable balance between efficacy (5.8%) and specificity (277) for onco-tools containing 3 pH-switches. Still further, calculated values in Figure 12d suggest that a pH-switch with a pKa of 6.4 should provide a desirable balance between efficacy (6.3%) and specificity (915) for onco-tools containing 4 pH-switches.

In this context, it should be appreciated that incorporation of an internal acid-specific H-bond in a pH-switch, as described in the preceding section, provides a suitable means for adjusting the pKa value of the pH-switch to a range appropriate for obtaining such dramatically improved specificities in these multi-pH-switch onco-tools.

The foregoing discussion and calculations focused on optimizing the pKa value of the pH-switches (via incorporation of an internal acid-specific H-bond) of an onco-tool as a means for achieving a desirable balance between efficacy and specificity (Figures 11 and 12). However, it should also be appreciated that increasing the partitioning coefficient (ie., the lipophilicity) of the onco-tool should have a similar effect on the balance between efficacy and specificity of multi-pH-switch onco-tools. This is because, as shown in Figure 4, such an increase in lipophilicity also serves to increase the pH at which the onco-tool can enter a lipophilic phase, such as a cell membrane. Accordingly, in this multi-pH-switch strategy for increasing an onco-tool's specificity for tumors one can adjust the balance between efficacy and specificity by adjusting the lipophilicity of the onco-tool, or by adjusting the pKa of the pH-switches (via incorporating an internal

acid-specific H-bond), or by a combination of adjusting the lipophilicity of the onco-tool and adjusting the pKa of the pH-switch components.

At this point it is appropriate to ask: do these calculated specificity values for multi-pH-switch onco-tools truly reflect that onco-tool's ability to selectively enter a lipophilic phase (such as octanol or a cell membrane) at the pH achievable in acidic areas of a tumor (pH 6.4), while largely avoiding entry into such a lipophilic phase at the pH of normal tissues (pH 7.4) ?

To address this question, a representative composition containing two pH-switch components, shown in Figure 13a, was synthesized. After synthesis, this composition was partitioned between n-octanol and aqueous buffers ranging from pH 5.6 to pH 8.0. The results of this partitioning experiment are plotted in Figure 13b. These results show that at pH 6.4 (achievable in tumors) about half of this composition partitioned into the octanol phase, while at pH 7.0 only about 2% partitioned into the octanol phase, and at pH 7.2 none of the composition was detected in the octanol phase.

As suggested by the calculated values plotted in Figure 12, these partitioning results likewise suggest that the majority of a composition containing two pH-switch components can exist in its non-ionic form (i.e., octanol-soluble form) at the pH which is present in acidic areas of tumors, and then that composition can switch almost completely to its anionic form (i.e., buffer-soluble) at the pH present in normal tissues. This provides experimental support for the value of the novel strategy of incorporating multiple pH-switch components into an onco-tool as a means for dramatically increasing the onco-tool's specificity for acidic areas of tumors.

A variety of representative multi-pH-switch onco-tool structures will be illustrated later in Section A.3. herein.

2. Cargo Component

a) Structural requirements

The cargo component is a structural component of an onco-tool which serves to incorporate a radioisotope whose emission is effective to report the presence of the onco-tool, or is effective to kill cells. The cargo component should satisfy the following three design requirements.

i) The cargo component in its precursor form should be effective to readily and efficiently incorporate, with minimal manipulations, its selected radioisotope.

ii) The radioisotope that is bound to the cargo component in its final form should remain so bound during the course of the diagnostic procedure or through the course of the therapeutic process wherein emissions from the radioisotopes are killing cells of the tumor.

iii) The cargo component in its final form, which includes a bound radioisotope, should be sufficiently small and of such a composition that it does not have an undue impact on the pH-dependent hydrophilicity/lipophilicity properties of the onco-tool. Stated differently, if the final form of the cargo component contributes excessive hydrophilicity it can suppress entry of the non-ionic form of the onco-tool into cells in acidic areas of tumors - thereby reducing efficacy. Conversely, if the final form of the cargo component contributes excessive lipophilicity it can cause undue sequestering in normal tissues - thereby reducing specificity.

Based on these requirements for the cargo component, it appears that radiohalogens selected from F, Br, I and At constitute the best radioisotope types, and that a vinyl group or a single unsaturated ring or single aromatic ring will best serve for binding the radiohalogen. Figure 14 illustrates a number of prospective cargo components in both selected precursor forms and in their final radioisotope-containing forms ready for diagnostic or therapeutic use.

b) Precursor and final forms

When the selected radioisotope which is to be bound to an onco-tool has a short half-life it is often desirable to make, ship, and store the onco-tool in its precursor form, and then to add the radioisotope cargo shortly before delivering the onco-tool into the subject to be diagnosed or treated. While a large number of different structures have been reported in the nuclear medicine field suitable for binding a wide variety of radioisotopes, many such structures are inappropriate for use in onco-tools. Figure 14 shows a few selected cargo components, in both their precursor forms and their final forms, that satisfy the particular requirements for use in onco-tools. Figure 15 shows synthetic schemes for preparing two such cargo components in their precursor forms.

These syntheses utilize key reactions for adding a tri-alkyl tin moiety which have been described by: Thibonnet, et al., Tetrahedron Letters, Vol. 39, page 4277 (1998); Miyake & Yamamura, Chemistry Letters, pages 981 - 984 (1989); Marshall & Bourbeau, Tetrahedron Letters, Vol. 44, pages 1087 - 1089 (2003); and, Corriu, Geng, & Moreau, J. Org. Chem. Vol. 58, page 1443 (1993). Figure 15 also illustrates a simple procedure for converting the precursor forms to their final radioisotope-containing forms, previously described by Zalutsky, page 96 of Chapter 4 titled: Radiohalogens for Radioimmunotherapy, in the book: Radioimmunotherapy of Cancer, Ed. by Abrams and Fritzberg, Pub. by Marcel Dekker, Inc. (2000). Procedures for preparing other suitable cargo components and incorporating the radioisotope to give the final form are described in: Zalutsky et al., Proc. Nat. Acad. Sci. USA, Vol. 86, Pages 7149 - 7153 (1989); and, Vaidyanathan & Zalutsky, Nature Protocols, Vol. 1, Pages 1655 -1661 (2006).

c) Selection of radioisotope cargo

It should be noted that it is the bound radioisotope of the onco-tool which determines the application of that onco-tool. If the bound radioisotope emits a signal which is readily detectable outside the body then that onco-tool can serve to detect tumors containing acidic areas. Conversely, if the radioisotope has an emission which is effective to kill cells then that onco-tool can serve for the treatment of tumors containing acidic areas. Furthermore, if the onco-tool contains a radioisotope, such as Iodine-131, which both emits a signal which is readily detectable outside the body (eg., gamma ray) and has an emission which is effective to kill cells (eg., beta particle) then that onco-tool can serve both for diagnosis and treatment of tumors containing acidic areas.

i) for detecting tumors

In onco-tools used for detecting tumors one has considerable latitude in selecting the radioisotope which is to generate the signal suitable for detection outside the subject. Several radioisotopes with favorable properties for diagnostic application include:

Radioisotope	Half-life
Fluorine-18	1.8 hours
Bromine-75	1.7 hours
Bromine-76	16 hours
Bromine-77	2.4 days
Iodine-123	13 hours
Iodine-124	4 days
Iodine-125	60 days
Iodine-126	13 days
Iodine-131	8 days

ii) for treating tumors

Successful treatment of a tumor faces two challenges. One challenge is to completely kill all the treatment-sensitive fast-dividing tumor cells at near normal pH close to tumor capillaries. The other more forbidding challenge is to completely kill all of the treatment-resistant quiescent tumor cells in acidic areas of the tumor. Therapeutic onco-tools were initially devised solely as a means for killing treatment-resistant quiescent cells in acidic areas of tumors. This was because onco-tools are designed to be sequestered only in acidic areas of tumors, and so it was presumed that such onco-tools would have to be used in conjunction with more conventional cancer therapies, such as radiation or chemotherapy, where the conventional therapies would serve for killing the treatment-sensitive fast-dividing tumor cells in more neutral areas of tumors closer to capillaries - because such areas would be largely devoid of onco-tools.

More recently the inventor has devised a dual-radioisotope strategy wherein onco-tools alone can be used to destroy the entire tumor, thereby obviating the need for co-treatment with more toxic and less specific conventional cancer therapies. This dual-radioisotope strategy entails using at least two onco-tool formulations effective to kill cells.

The onco-tool formulation used to kill the treatment-resistant quiescent tumor cells should contain a radioisotope which emits high linear energy transfer radiation in order to kill all of the radiation-resistant quiescent tumor cells in or near which radioisotope-carrying therapeutic onco tool is positioned.

One option for achieving this demanding requirement is to use a

radioisotope which emits an alpha particle that releases a vast amount of energy over a very short distance (a few cell diameters), such that the released energy is highly effective to kill even cells which are highly resistant to most radiation. The best radioisotope for this purpose appears to be the radiohalogen, Astatine 211. This radioisotope is generated from natural Bismuth 209 in a medium-energy cyclotron equipped with an alpha particle beam. Astatine-211 has a half-life of 7.2 hours and emits two alpha particles with energies of 5.87 and 7.45 million electron volts, which have been shown to devastate cells within the approximately 50 to 80 micron path lengths of the emitted alpha particles (a few cell diameters). Just a few such alpha emissions can kill even the most radiation-resistant quiescent tumor cell.

Another class of radioisotopes which emit high linear energy transfer radiation are those which emit Augar and/or Coster-Kronig electrons. Such radioisotopes, if positioned within the cell to be killed, can cause devastating damage to the cell's DNA and so are extremely cytotoxic when localized in close proximity to the cell nucleus. Radioisotopes of this type which are particularly suitable for therapeutic onco-tools include: Bromine-77 (half-life of 57 hours) and Iodine-123 (half-life of 13 hours).

The onco-tool formulation used to kill the treatment-sensitive fast-dividing tumor cells closer to capillaries should contain one or more radioisotopes which emit particles that release sufficient energy along their path to kill treatment-sensitive fast-dividing tumor cells. More importantly, those emitted particles must have a sufficiently long path length that they are effective against cells up to several hundred microns from where the onco-tool is sequestered - allowing them to kill the treatment-sensitive fast-dividing tumor cells in more neutral areas of the tumor, which can be up to several hundred microns from acidic areas where onco-tools are sequestered. Beta-emitting radioisotopes best satisfy this requirement. While there are a number of beta-emitting radioisotopes which can be used in this dual-isotope therapy strategy, the following radiohalogens have favorable properties for this application, including mean path lengths of greater than 600 microns.

Radioisotope	Half-life
Bromine-82	36 hours
Bromine-83	2.4 hours
Iodine-126	13 days
Iodine-130	12 hours
Iodine-131	8 days
Iodine-132	2.3 hours
Iodine-133	21 hours
Iodine-135	7 hours

Compared to treatment where an onco-tool is used only to kill the quiescent cells in acidic areas of tumors, and is combined with conventional cancer therapies effective to kill the fast-dividing tumor cells in other areas of tumors having a higher pH, it is expected that this new dual-radioisotope onco-tool therapy strategy wherein onco-tools alone are used to kill the entire tumor, will afford a simpler, less costly, far less toxic, and far more effective treatment.

3. Onco-Tool Structures

a) Structural requirements

Each onco-tool must contain two or more pH-switch components. This "two or more" requirement is essential in order to achieve a specificity which is substantially greater (at least over 20, and preferably over 40) than is typically provided by current cancer therapies (specificities estimated to be typically in the range of about 2 to 8). Further, each onco-tool must have a structure such that at pH 7.4 it exists almost completely in an anionic hydrophilic form, but at pH 6.4 a significant portion (preferably 1% or more) shifts to a non-ionic lipophilic form effective to be sequestered in acidic areas of tumors. Each onco-tool must also contain a cargo component which is effective to bind a radioisotope, or which contains a radioisotope suitable for reporting the presence of the onco-tool and/or suitable for killing cells.

The following three sections describe onco-tools with 2, 3, and 4 pH-switches. With respect to such onco-tool structures, it should be appreciated that any selected combination of components will generally require optimization of the R groups in order to achieve adequate efficacy and a desirable balance between

efficacy and specificity. Procedures for such optimizations of the R groups are described and illustrated later herein in Section B and in Figures and Examples relating to that section.

b) Onco-tools with 2 pH-switches

These onco-tools have the simplest structures and are generally the easiest to synthesize. They typically have specificity factors potentially ranging from about 20 to about 80. Figure 16 illustrates a variety of representative two-pH-switch onco-tools which satisfy the key structural requirements for onco-tools. Figure 16a illustrates onco-tools containing two simple pH-switches. Figure 16b illustrates onco-tools containing two advanced pH-switches. Figure 16c illustrates onco-tools containing two advanced pH-switches designed to form low-barrier H-bonds.

c) Onco-tools with 3 pH-switches

Onco-tools containing 3 pH-switches are more complex than the 2-pH-switch onco-tools described above, and are generally more challenging to synthesize. However, the 3-pH-switch onco-tools have the merit of affording appreciably higher specificity factors, potentially ranging from about 100 to about 500. Figure 17 illustrates a variety of representative three-pH-switch onco-tools which satisfy the key structural requirements for onco-tools. Figure 17a illustrates onco-tools containing three advanced pH-switches and Figure 17b illustrates onco-tools containing three advanced pH-switches designed to form low-barrier H-bonds.

d) Onco-tools with 4 pH-switches

Onco-tools containing 4 pH-switches are even more complex than the 3-pH-switch onco-tools described above, and are generally still more challenging to synthesize. However, the 4-pH-switch onco-tools have the potential for even higher specificity factors, potentially ranging from about 400 to about 2500. Figure 18 illustrates two representative onco-tools which satisfy the key structural requirements for onco-tools and contain four advanced pH-switches designed to form a low-barrier H-bond.

B. Synthesis, Testing, And Optimization Of Components And Onco-Tools

1. Preparation of structures containing representative pH-switches

Before beginning the development of any new onco-tool type it is generally desirable to first prepare and assess the properties of a variety of pH-switch types, as well as variations within a type wherein variations of the R group serve to impart a wide range of lipophilicities to the structures.

a) Simple pH-switches

Figure 19 illustrates a representative synthetic route for onco-tool structures containing simple pH-switches, with lipophilicity adjusted by varying the R group.

b) Advanced pH-switches

Figure 20 illustrates a synthetic route for structures containing an advanced pH-switch type, with lipophilicity adjusted by varying the R1 group.

c) Advanced pH-switches designed to form a low-barrier H-bond

Figures 21 and 22 illustrate synthetic routes for structures containing a number of advanced pH-switch types designed to form a low-barrier H-bond.

Figure 21 shows useful amine-ester and ketone-ester intermediates for preparing pH-switches and for preparing onco-tools. Synthetic procedures for making key core components of such intermediates have been reported in the following sources: 1) Goldman, Jacobsen, and Torssell, Synthesis in the Camphor Series. Alkylation of Quinones with Cycloalkyl Radicals. Attempted Synthesis of Lagopodin A and Desoxyhelicobasidin. Acta Chemica Scandinavica B 28 (1974) 492 -500; and, 2) Brian Thomas Connell, Synthesis and Evaluation of a New Camphor-Derived Lactam as a General Chiral Auxiliary for the Asymmetric Diels-Alder and Aldol Reactions. Thesis submitted to the Dept. of Chemistry, University of Rochester, Rochester, New York (1995).

Figure 22 then illustrates representative synthetic routes for converting several such intermediates to various pH-switches designed to form an internal acid-specific low-barrier H-bond.

2. Testing pH-switches

Simple titration assays provide useful information about the pH-dependent solubility properties of pH-switch structures, as well as their pKa values. Example 7 describes titration procedures which have been used for studying pH-switch components. Figure 23 shows representative results from a variety of past titrations.

Figure 23a shows a conventional titration curve, where the titration was carried out in Methanol/Water, 1:1 by vol., for a simple carboxylic acid (Butyric acid) and an advanced pH-switch (an acid-amide derivative of Camphoric acid). In the companion plot the same titration results are plotted in the more informative first derivative form.

Figure 23b shows conventional titration curves, where the titration was carried out in water and each specie was present at 33 milliMolar concentration. Titration results are shown for three related two-pH-switch onco-tools (but with stable Iodine instead of radioactive Iodine) which vary only in their R group. Titration results are also shown for a related three-pH-switch structure where the R group is methyl.

Figure 23c shows a titration curve, plotted as the first derivative, for an advanced pH-switch designed to form a low-barrier H-bond. This titration was carried out in water and the pH-switch, comprising an N-oxide/acid structure derived from Camphoric acid, was present at a 5 milliMolar concentration.

Figure 24 shows experimentally-determined pKa values for three pH-switch structures, including: an amide/acid advanced pH-switch derived from Camphoric acid; an N-oxide/acid advanced pH-switch designed to form a low-barrier H-bond, derived from Isonipocotic acid; and, an N-oxide/acid advanced pH-switch designed to form a low-barrier H-bond, derived from Camphoric acid (also shown as structure i of Figure 10c, and the structure shown in Figure 23c).

3. Preparation of representative onco-tools and conversion to final form

a) Onco-tools with simple pH-switches

Figure 19 illustrates a synthetic route for preparing a representative onco-tool containing simple pH-switches.

b) Onco-tools with advanced pH-switches

Figure 25 illustrates synthetic schemes for representative onco-tools containing advanced pH-switches. Figure 25a shows an onco-tool wherein its two pH-switches are joined by a di-acylhydrazide structure. Figure 25b shows an onco-tool wherein its two pH-switches are joined by a di-amide structure.

c) Onco-tools with advanced pH-switches designed to form a low-barrier H-bond.

Figure 26 illustrates synthetic schemes for representative onco-tools containing advanced pH-switches designed to form a low-barrier H-bond.

Figure 26a shows a synthetic scheme for a 2-pH-switch onco-tool wherein a single N-oxide moiety serves as the H-bond acceptor moiety for two carboxylic acid H-bond donor moieties. Figure 26b shows a synthetic scheme for a 2-pH-switch onco-tool wherein both of the nitrogens of a hydrazine moiety serve as the H-bond acceptor moieties for two carboxylic acid H-bond donor moieties. Figure 26c shows a synthetic scheme for a 2-pH-switch onco-tool wherein cyanomethyl amine moieties serve as the H-bond acceptor moieties for the carboxylic acid H-bond donor moieties. Figure 26d shows a synthetic scheme for a 4-pH-switch onco-tool wherein each N-oxide moiety serves as the H-bond acceptor moiety for two carboxylic acid H-bond donor moieties.

It should be noted that in the foregoing syntheses of onco-tools containing advanced pH-switches designed to form a low-barrier H-bond, when a ketone/ester intermediate, such as structures b, d, and f or Figure 21, is used to reductively alkylate an amine/ester wherein the amine moiety is cis to the ester moiety, such as structures a, c, and e of Figure 21, generally the resulting 2-pH-switch product comprises 2 isomers, one where both of the pH-switch components are cis with respect to the amine and ester, and the other product where one of the pH-switch components is cis and one of the pH-switch components is trans with respect to the amine and ester moieties. In such cases it is desirable to purify the desired cic/cis product, and discard the cis/trans product. One way to accomplish this is to exploit the fact that the trans pH-switch component cannot form an internal acid-specific low-barrier H-bond. As a consequence, its pKa will typically be approximately 5.0 in an aqueous solution.

In contrast, the cis pH-switch component generally can form the desired internal acid-specific low-barrier H-bond, which typically raises the pKa of the contained carboxyl moiety to about 6.0 or higher. As a consequence, after the ester moieties have been cleaved to give carboxyl moieties the desired cis/cis form should generally be readily separable from the undesired cis/trans form by partitioning between an organic phase, such as Tetrahydrofuran, t-Butylmethylether, or Dichloromethane, and an aqueous phase buffered at round 6.0. In such a partitioning system the desired cis/cis form should generally preferentially partition into the organic phase, while the undesired cis/trans form should preferentially partition into the aqueous buffer phase.

The same basic separation strategy can also be used to separate the desired all-cis form of the 4-pH-switch onco-tool of Figure 26d from the multiple forms containing at least one trans pH-switch.

4. Testing and optimization of onco-tools

The following sources of information serve as a starting point for designing and developing an effective and specific onco-tool. The efficacy and specificity plots in Figure 12 provide guidance on desirable pKa values for the pH-switch components as a function of how many pH-switch components the selected onco-tool is to contain. Figure 4 provides guidance as to the impact of changing the partition coefficient of the non-ionic form of the onco-tool - where the partition coefficient is typically adjusted by changing the lipophilicity of the R groups of the pH-switch components. Furthermore, synthesis of various prospective pH-switch types and their R-group variants, such as described in Section B.1. above, and subsequent titration studies of those pH-switches, as described in Section B.2. above, provide valuable information for selecting suitable pH-switch components for a prospective onco-tool. Finally, Figure 14 illustrates a number of cargo components suitable for incorporation into onco-tools.

While the foregoing information on the component parts provide a valuable basis for the design and development of an effective and specific onco-tool, nonetheless, once those component parts are assembled into a prospective onco-tool in its radioisotope-containing form, in order to achieve adequate efficacy and a desirable balance between efficacy and specificity it is generally necessary to optimize that onco-tool structure.

a) Iterative optimization process: synthesis and testing in cultured cells A preferred optimization process entails the following:

i) Prepare set of onco-tools varying in lipophilicity

The optimization process is begun by preparing a set of prospective onco-tool structures incorporating the selected components, wherein onco-tools of the set exhibit a wide range of lipophilicities due to their containing different R1 and R2 groups. Each onco-tool of the set should also contain a suitable radioisotope, such as Iodine-131, which affords easy detection and quantitation in biological systems.

ii) Assess entry into cultured cells at pH 6.4 and pH 7.4

Each of those prospective onco-tool structures should be tested in a relatively simple biological system wherein the prospective onco-tool is exposed to the principal biological environments and structures it will encounter in a living subject. As described in Example 8, a preferred biological system for such initial testing comprises mammalian cells cultured in serum-containing medium buffered at pH 7.4 to emulate normal tissues, and buffered at pH 6.4 to emulate acidic areas of tumors. Briefly, two different wells of cultured cells are exposed for one hour to a given onco-tool. In one culture well the onco-tool is in pH 6.4 medium. In the other culture well the onco-tool is in pH 7.4 medium. After incubating 1 hour at 37 deg. C, the onco-tool-containing medium is removed and the cells are washed thoroughly with medium of the same pH, and then radioisotope retained by the cells is counted to provide a measure of the relative quantity of onco-tool which has been sequestered under each of the two pH conditions.

The preferred onco-tool structures are those which are maximally sequestered by the cells at pH 6.4, but minimally sequestered by the cells at pH 7.4.

The first application of the above two optimization steps should generally serve to identify a reasonably narrow range of onco-tool lipophilicities which give acceptable efficacy (related to amount sequestered at pH 6.4) and specificity (related to the ratio: amount sequestered at pH 6.4 / amount sequestered at pH

7.4) values. Alternatively, such initial testing may instead serve to indicate that the selected onco-tool components are inadequate and different pH-switch components and/or a different cargo component should be used in one's prospective onco-tool structures.

iii) Repeat above steps as needed

Additional optimization cycles within progressively narrower structural ranges may lead to still better onco-tool activity.

b) Assess disposition in normal mice

The above cell culture assays are relatively fast, simple, provide quantitative results, and are amenable to initial testing of a substantial number of prospective onco-tool structures. However, it should be appreciated that this initial cell culture screening system does not perfectly emulate the true complexity of a living subject. Accordingly, it is desirable to next take a reasonable number of the most promising prospective onco-tool structures, identified in the above iterative optimization process, and test them in living mammals. As a first step in such animal testing, it is useful to test these promising onco-tool structures in normal mice (pre-treated to assure that their urine is slightly basic; see Section C.7. below), as described in Example 9. Such tests in normal mice allow one to discard any onco-tools which are found to exhibit an excessive affinity for normal tissues.

c) Assess disposition in tumor-bearing mice

While the above relatively simple tests in normal tumor-free mice allow one to discard those onco-tools which have an excessive affinity for normal tissues, the more decisive test for a prospective onco-tool structure is to test it in tumor-bearing mice, as described in Example 10. Briefly, this entails injecting (preferably intravenous) each of the Iodine-131-containing onco-tools into several tumor-bearing mice, and then waiting a suitable period of time (on the order of 5 to 24 hours) to allow normal excretion by the kidneys of that portion of the administered dose which is not sequestered in tissues and/or tumors of the mice. One then terminates the mice and excises the major organs and any obvious tumors, followed by quantitating the radiation emissions from the excised organs,

tumors, and remaining carcass.

Onco-tools will work best when two ancillary methods are used. One such method entails pre-treating the mice to prevent re-uptake of onco-tool into the cells lining the proximal tubules of the kidneys. This re-uptake is blocked by rendering the urine slightly basic, as described in Section C.7. below. The other method entails pre-treating the mice to further increase the acidity (reduce the pH) in hypoxic/acidic areas of their tumors. Three pre-treatments for this purpose are described later herein in Section C.5. At least one, and preferably a combination of two or three such pre-treatments should be employed in order to adjust the tumor micro-environment so as to be best suited for effective and specific onco-tool activity.

d) Preclinical and clinical testing

Procedures for testing radioisotope-containing substances in live animals, including humans, are well known in the nuclear medicine field, and particularly in the sub-field of radio-immunotherapy. Such known methods can be readily adapted for testing onco-tools by incorporating the methods of using onco-tools described in Section C. below.

C. Methods Of Using Onco-Tools

1. Diagnostic Method

Because onco-tools exploit the acidity which is a near-universal characteristic of tumors, onco-tools should be effective to detect most or all types of tumors with sizes ranging from near-microscopic to very large. The following method of using onco-tools for detecting tumors is suitable for many research applications, as well as for both veterinary medicine and human medicine. The diagnostic method generally includes, but is not limited to, the following steps:

Step 1. Provide a diagnostic onco-tool in its final form - either by contacting the precursor form of the onco-tool with a suitable radioisotope which is effective to report its presence within a tumor to a detector outside the living subject, or by obtaining directly from a supplier the final form of a diagnostic onco-tool already containing such a radioisotope.

Step 2. Deliver that diagnostic onco-tool into the subject - typically by intravenous injection.

Step 3. Wait a suitable period of time for onco-tool to be sequestered in acidic areas of any tumors which may be present (eg., from about 10 minutes to about 50 minutes). Considerably increased sensitivity can be obtained by waiting additional time (hours) for excretion through the kidneys of most of that portion of the onco-tool dose which has not been sequestered in acidic areas of tumors. Waiting this additional time serves to greatly lower background signal from normal tissues and allow detection of even quite small tumors. In this regard, over the course of a few hours most of the injected dose of onco-tool should be excreted by the kidneys, with significant retention of onco-tool only occurring if one or more tumors are present. It is expected that typically in subjects who do not have tumors most of the onco-tool will be excreted in less than 24 hours, and probably in less than about 4 hours. The rate of excretion of non-sequestered onco-tool can be increased by increasing the subject's fluid intake, particularly if that fluid contains a diuretic.

Step 4 The final step in the diagnostic method is to scan the subject with equipment suitable for detecting the emission from the radioisotope component of the onco-tool in order to assess if significant onco-tool has been sequestered in one or more tumors. With modern imaging equipment, such as gamma ray scanners and PET scanners, tumors should show up as an obvious radioisotopic hot spot at the site of each tumor.

2. Therapeutic Method

Because onco-tools exploit the acidity which is a near-universal characteristic of tumors, onco-tools should be effective to treat most or all types of tumors with sizes ranging from near-microscopic to very large. The following methods of using onco-tools for treating tumors are suitable for many research applications, as well as for both veterinary medicine and human medicine. The therapeutic methods generally include, but are not limited to the following.

a) Single-radioisotope method

If a subject has been found to have one or more tumors, those tumors can be treated with a single therapeutic onco-tool containing a radioisotope effective

to kill cells. The therapeutic method generally includes, but is not limited to, the following two steps:

Step 1. Provide a therapeutic onco-tool in its final form containing a radioisotope effective to kill cells. This can be done either by contacting the precursor form of the onco-tool with a suitable radioisotope, or by obtaining directly from a supplier the final form of the therapeutic onco-tool already containing such a radioisotope.

Step 2. Deliver that therapeutic onco-tool into the subject - typically by intravenous injection.

In this therapeutic method the radioisotope effective to kill cells can be either one whose emission has a high linear energy transfer, such as an alpha particle, or one which emits a beta particle. The particular merit of using a radioisotope having a high linear energy transfer emission, such as the alpha-emitting radioisotope Astatine-211, is that it is appreciably more effective than beta-emitting radioisotopes for killing the quiescent cells in acidic areas of tumors. However in regard to using onco-tools containing such radioisotopes, their emissions generally have a quite short path length (eg., 80 microns or less). Thus, it may be necessary to use such onco-tools in combination with conventional cancer therapies in order to also assure the destruction of the fast-dividing tumor cells in areas of more normal pH near capillaries. Further, it should be appreciated that the preferred alpha emitter, At-211, has a short half-life (7 hours) and so shipping this radioisotope, or an onco-tool containing this radioisotope, a substantial distance from the site where it is generated can be a problem. Still further, At-211 must be generated in a high-end cyclotron with an alpha beam capability, and at present there are only relatively few sites where such equipment is available.

An alternative single-radioisotope method entails providing a therapeutic onco-tool containing a beta-emitting radioisotope. While beta emissions are significantly less effective than alpha emissions for killing the quiescent cells in acidic areas of tumors, this lesser effectiveness of beta emissions can be compensated for by using a much greater dose. While this much greater dose will compromise specificity somewhat, nonetheless, it has the potential to give acceptable therapeutic results because of the exceptionally high level of specificity for tumors achievable by onco-tools. In this regard, the beta emitter,

Iodine-131, has several useful properties: it is readily available in large quantities at a moderate price; because of its relatively long half-life (8 days) it can be shipped and stored for reasonable periods of time before use; and, while it has only a moderate mean path length (about 900 microns), nonetheless that is sufficient to kill cells throughout the tumor, including the fast-dividing tumor cells in regions closer to neutral pH near capillaries - which will be relatively devoid of sequestered onco-tool.

b) Dual-radioisotope method

As noted in earlier herein, radioisotopes which have high linear energy transfer emissions are optimal for killing the radiation-resistant quiescent cells in acidic areas of tumors, but the short path length of such emissions makes an onco-tool containing such radioisotopes rather ineffective for killing the fast-dividing cells in distal areas of tumors near capillaries where the pH is closer to neutral - because such areas will be relatively devoid of sequestered onco-tool.

Conversely, an onco-tool containing a beta-emitting radioisotope is relatively ineffective against the radiation-resistant quiescent cells of the tumor, but that onco-tool containing the beta-emitting radioisotope can be quite effective for killing the radiation-sensitive fast-dividing cells in areas of tumors where the pH is closer to neutrality - even when that onco-tool is only present in the acidic areas of the tumor. This is because the greater path length of the beta particles allow them to reach and kill those more sensitive tumor cells.

Accordingly, a preferred method for treating tumors is to use a combination of two therapeutic onco-tools, where one onco-tool contains a radioisotope having a high linear energy transfer emission (preferably selected from Astatine-211, Bromine-77, and Iodine-123) to kill the proximal radiation-resistant quiescent cells in acidic areas of the tumor, and the other onco-tool contains a beta-emitting radioisotope to kill the distal radiation-sensitive fast-dividing cells in higher-pH regions near tumor capillaries, which are relatively devoid of sequestered onco-tool.

3. Comprehensive Method For Detecting And Treating Tumors

Onco-tools offer the highly desirable properties of being able to both detect and treat most or all types and sizes of tumors, ranging from near-microscopic to

very large. Because the diagnostic and the therapeutic onco-tools can be virtually identical - but typically differing only in the contained radioisotope - in general if a given onco-tool structure is effective to detect a tumor, then that same onco-tool structure, but typically with a different radioisotope, should also be effective to treat that same tumor. These special properties of onco-tools facilitate a comprehensive method for detecting tumors in living subjects, followed by treatment of any tumors so detected. This comprehensive method is suitable for both veterinary medicine and human medicine. It includes, but is not limited to, the following steps.

a) Detecting tumors

Step 1 The first step it to provide a diagnostic onco-tool in its final form containing a radioisotope which is effective to report its presence within a tumor to a detector outside the living subject.

Step 2. The next step is to deliver that diagnostic onco-tool into the subject - typically by intravenous injection.

Step 3. The subsequent step is to wait a suitable period of time for onco-tool to be sequestered in acidic areas of any tumors which may be present. This step may also include waiting additional time for excretion through the kidneys of most of that portion of the onco-tool dose which has not been sequestered in acidic areas of tumors. During this period of time the subject may also be given fluid, particularly fluid that contains a diuretic, to increase the excretion of that portion of the onco-tool dose which has not been sequestered in acidic areas of tumors.

Step 4 The last step in the detection process is to scan the subject with equipment suitable for detecting the emission of the radioisotope of the onco-tool in order to assess if significant onco-tool has been sequestered in one or more tumors. With modern imaging equipment, such as gamma ray scanners and PET scanners, tumors should show up as an obvious radioisotopic hot spot at the site of each tumor.

In the event one or more tumors are detected in step 4, one then proceeds to treat the detected tumors.

b) Treating detected tumors

Step 5. To treat the detected tumor, one or more therapeutic onco-tools are provided. While this can be a single onco-tool containing a beta-emitting radioisotope, it is generally preferred to provide two or more onco-tools, where one contains a radioisotope which emits high linear energy transfer radiation, and another contains a beta-emitting radioisotope.

Step 6. The one or more provided onco-tools are delivered into the subject - generally by intravenous injection.

4. Strategy For Dealing With Micro-Metastases

When a tumor reaches a substantial size (such as one centimeter or larger) it commonly begins to metastasize, wherein single tumor cells or very small aggregates of tumor cells are released from the parent tumor, and those released cells can colonize at distant sites in the body. These colonies of cells, called micro-metastases, can then grow into new progeny tumors. The difficulty this presents for the onco-tool therapy method is that in the period of time between formation of the micro-metastases and the time it takes such micro-metastases to grow to a size (about 1 millimeter in diameter) sufficient to generate their own acidic areas, those sub-millimeter progeny tumors typically cannot be detected or killed by onco-tools. Thus, while the parent tumor containing acidic areas can be detected and destroyed by onco-tools, any progeny micro-metastases smaller than about 1 millimeter in diameter are expected to survive the onco-tools treatment and ultimately lead to a relapse - though such a relapse may not occur for several years after the initial onco-tool treatment.

A strategy for solving this micro-metastases problem is to wait a period of time after the initial onco-tool therapy (steps 5 and 6 in Section 3 above) sufficient for any micro-metastases, that might be present and survived the initial treatment, to grow to a size where they develop acidic areas (typically about 1 millimeter in diameter). Conversely, those progeny micro-metastases should not be allowed to grow to the much larger size (probably on the order of 1 centimeter in diameter) where they too begin to metastasize. Once micro-metastases that might have escaped the first onco-tool treatment are in this proper size range (large enough to contain acidic areas, but not so large as to have begun

metastasizing), one again carries out the onco-tool detection and treatment process (repeat of steps 1 through 6 in Section 3 above).

A complication in the above strategy is that tumors exhibit a wide range of growth rates, and so it is difficult to predict how long it will take for any micro-metastases which might have escaped the first treatment to reach a size where they contain acidic regions. Therefore, the prudent course is to repeat diagnostic steps 1 through 4 above at appropriate intervals (perhaps once a year) continuing for a sufficient length of time (perhaps 4 to 6 years) to virtually assure that if any micro-metastases did escape destruction in the initial onco-tool therapy, then such micro-metastases would have grown to a size sufficient to generate acidic regions and so be detected in one of the subsequent repeat diagnostic procedures (repeats of steps 1 through 4 above). If and when one of the repeat diagnostic procedures does detect one or more tumors, then the patient would be again treated as per steps 5 and 6 above. It seems likely that such a second treatment has a high probability of completely destroying any progeny tumors which might have escaped in the form of micro-metastases during the course of the initial onco-tool treatment - thereby completely clearing the patient of the original tumor and all its progeny.

5. Treat To Decrease pH In Tumors For Increased Efficacy And Specificity

Both the efficacy and the specificity of an onco-tool for acidic areas of tumors is a strong function of the pH differential between the acidic areas of the tumor and normal tissues. Thus, if one can selectively reduce the pH in acidic areas of tumors even further than is found in the natural condition - without concomitant reduction of the pH in normal tissues, this will afford greater sensitivity in diagnostic applications and greater efficacy and specificity for therapeutic applications of onco-tools.

Over the past half century a number of treatments have been reported to alter the pH in tumors - some causing the pH to increase and some causing the pH to decrease. In the context of diagnostic and therapeutic onco-tools, it is the treatments that selectively decrease the pH in tumors, without a concomitant reduction in the pH in normal tissues, which are of interest. Following are three such treatments that have been reported to selectively reduce the pH in tumors.

a) It has long been known that introduction of glucose into tumor-bearing animals acts to reduce the pH in the interstitial space in hypoxic areas of the tumors, typically for a period of about 2 to 3 hours, while having little or no effect on the pH of the interstitial space in normal tissues (Naeslund & Swenson (1953) Acta Obstet. Gyneocol. Scand. 32, 359 - 367). Additional intake of glucose by mouth can significantly extend the length of time during which the pH in tumors remains so reduced.

b) The pH in acidic areas of tumors can also be further reduced by treating with the mitochondrial inhibitor, meta-iodobenzylguanidine, again apparently without undue effect on the pH in normal tissues (Jahde et. al., (1992) Cancer Research 52, 6209 - 6215).

It has also been reported that the pH in acidic areas of tumors can be further reduced by as much as 0.7 pH unit by use of a combination of glucose and meta-iodobenzylguanidine (Kuin et al., (1994) Cancer Research 54, 3785 - 3792).

c) Still further, the pH in acidic areas of tumors can be further reduced by vasodilator drugs which are routinely used to treat persons with hypertension (Adachi and Tannock (1999) Oncology Research 11, 179 - 185). Such drugs are probably effective because the abnormal vasculature of tumors generally lacks vasoconstrictor muscle cells and their nerve fibers. Therefore, when a subject is given a vasodilator drug, resistance to blood flow is unchanged in tumors but decreases in normal tissues. These differential effects of the vasodilator result in a significantly greater blood flow through normal tissues and a concomitant reduction in blood flow through the tumor. In turn, this reduced blood flow through the tumor probably reduces the washout of the lactic acid produced by tumor cells in hypoxic areas of the tumor - resulting in the observed vasodilator-mediated drop in the pH in acidic areas of the tumor.

Such treatments, but preferably a combination of two or more such treatments to further reduce the pH in acidic areas of tumors should increase the sequestering of onco-tool in the now-more-acidic areas of the tumor, as well as lead to an increase in the areas of the tumor which are sufficiently acidic to

sequester onco-tool. Both of these effects serve to increase the efficacy and the specificity of the onco-tool.

Making the tumor more acidic also allows one to use an onco-tool with pH-switches having a lower pKa - which can result in a significant increase in the onco-tool's specificity.

6. Use Multiple Onco-tools Varying In pKa To Increase Efficacy And Specificity

Reported experimental measurements of the acidity in tumors have shown a gradient of pH ranging from about 7.0 close to tumor capillaries and decreasing with increasing distance from tumor capillaries, with values as low as about 6.0 having been reported for areas most distant from tumor capillaries. For tumors having a broad range of pH values there is the potential that therapeutic onco-tools with too high of a pKa may rapidly enter cells in mildly-acidic areas close to tumor capillaries to such an extent that inadequate onco-tool remains to reach the lowest pH areas of the tumor most distant from the capillaries. Alternatively, therapeutic onco-tools with too low of a pKa may be too poorly sequestered in mildly-acidic areas closer to capillaries in the tumor, thereby leading to inadequate treatment of such areas. One strategy for dealing with a wide pH range within a tumor is to use a combination of two or more onco-tools, where one onco-tool with a higher pKa is maximally effective in higher-pH regions of the tumor closer to capillaries, and where another onco-tool with a lower pKa is maximally effective in lower-pH regions of the tumor which are further from capillaries. The rationale for using such a combination of onco-tools is that the onco-tool with the higher pKa may be largely sequestered in areas close to capillaries - leaving little available for reaching areas of lower pH further from capillaries Conversely, the onco-tool with the lower pKa should be poorly sequestered in areas of higher pH near capillaries and so remain available to diffuse into areas of lower pH further from capillaries where they can then be effectively sequestered. Thus, together such a combination of low-pKa and high-pKa onco-tools should better achieve complete destruction of the tumor.

7. Treat To Increase pH In Urine To Protect Kidneys From Damage

One of the functions of the kidneys is to maintain the pH in the body at

very close to 7.4. To carry out this function the kidneys can excrete urine ranging from moderately basic to fairly acidic. In this process a substance is filtered from the blood in the glomerulus of the kidney, after which that substance (dissolved in urine) passes through the proximal tubule where critical components excreted in the glomerulus are reabsorbed by cells lining the proximal tubule. If the urine is acidic at this point then an onco-tool in this acidic environment has the potential of binding and entering the cells lining the proximal tubules of the kidneys, much as the onco-tool enters tumor cells from an acidic extracellular environment. Stated differently, if the excreted urine is sufficiently acidic the onco-tool will switch to its non-ionic lipophilic form. If that switch to a lipophilic form occurs in a region where the urine is in direct contact with cell membranes, such as is the case for cells lining the proximal tubules of the kidneys, then the onco-tool can enter such cells. In the case of a diagnostic onco-tool this entry of onco-tool into cells lining the proximal tubules can lead to excessive signal emanating from the kidneys, which could obscure a tumor near or in the kidneys. In the case of a therapeutic onco-tool this entry of onco-tool into cells lining the proximal tubules can lead to killing the cells lining the proximal tubules - effectively rendering the kidneys non-functional.

Luckily, there are methods well known in the medical arts for raising the pH of a subject's urine for sufficient time to carry out diagnosis or therapy with onco-tools. By using a suitable drug to maintain the urine at a slightly basic pH during the period when most of the non-sequestered onco-tool is being excreted by the kidneys, that onco-tool excreted in the urine will remain in its anionic hydrophilic form and be safely passed from the body by urination. One safe and effective substance for rendering the urine basic is the carbonic anhydrase inhibitor drug, Acetazolamide. Thus, a prudent course in any diagnosis or treatment with onco-tools is about one to two hours prior to delivering the onco-tool into the subject, first treat the subject with this drug, or other substance effective to raise and maintain the pH of the urine at a pH above about 7.4.

8. Monitor and Flush Bladder for Increased Safety

Because of the potential harm to the kidneys by therapeutic onco-tools if the kidneys are excreting acidic urine, it may also be desirable to continually monitor the pH of the urine entering the bladder by using a micro-pH-probe at the

end of a catheter. The preferred period of time for such monitoring is from just before injection of the onco-tool until such time as most of the onco-tool not sequestered in acidic areas of tumors has been excreted by the kidneys (typically about 3 to 6 hours). Such monitoring will allow emergency intervention (such as injecting an additional dose of Acetazolamide) in the event the pH of the newly excreted urine begins to drop below about pH 7.4.

Over the course of a few hours much of the injected dose of an onco-tool will be excreted by the kidneys and is stored in the subject's bladder until voided by urination. For the case of an onco-tool diagnostic application, this buildup of radioactive onco-tool in the bladder can obscure the presence of tumors in close proximity to the bladder. For the case of a therapeutic onco-tool the buildup of radioactive onco-tool in the patient's bladder has the potential of damaging the bladder. If buildup of radioactive onco-tool in the bladder proves to be a significant problem, it can be substantially reduced by using an inflow/outflow catheter to irrigate the bladder from the time the onco-tool is injected until such time as most of the injected dose has been cleared from the patient's body.

Irrigating of the bladder during the period of time when most of the onco-tool dose is being excreted by the kidneys serves an additional purpose, that being the solution carried out of the bladder can be passed into a shielded storage vessel where it can be contained until the radioisotope has decayed to a safe level (typically 10 half-lives of the radioisotope). This serves to assure the safety of the medical personnel attending the subject, and it largely precludes inadvertent contamination of lavatories and other areas by radioactive onco-tool voided by the subject.

EXAMPLES:

Example 1. Effect of lipophilicity on pH of transition between ionic and non-ionic forms

The sodium salts of propionic acid (a hydrophilic carboxylic acid) and octanoic acid (a lipophilic carboxylic acid) at a concentration of 33 milliMolar in water were titrated with 5 M HCl. The mid-point of the transition between salt and acid (pKa value) was found to be an expected 4.83 for propionic acid, but a

surprisingly-high 5.5 for octanoic acid. The first derivative of the titration curve was symmetrical for the propionic acid, but highly unsymmetrical for the octanoic acid. In addition, droplets of octanoic acid oiled out of solution during the course of the octanoic titration.

In contrast, when the salts of these same two acids were titrated in the semi-aqueous medium: methanol/water, 1:1 by vol., their mid-points of transition were seen to be virtually identical, and the first derivative of their titration curves were now both symmetrical. Finally, in the titration in the semi-aqueous medium, methanol/water, no octanoic acid oiled out of solution.

These results suggest that the surprisingly high mid-point in the titration seen for octanoic acid in aqueous solution was due simply to the solubility effects illustrated in Figures 3 and 4. The results further show that carrying out the titrations in the semi-aqueous solution, methanol/water, 1:1 by volume, avoids these solubility effects on the titration curves.

Example 2. Comparison of acid-specific and non-acid-specific internal H-bonds on pH of transition between ionic and non-ionic forms

Thirty milliMolar solutions of the sodium salts of compounds shown in Figure 6a (designed to form internal acid-specific H-bond) and 6b (designed to form non-acid-specific H-bonds) were titrated with 5 M HCl in aqueous solution. The first derivative of the titration curve for the compound in Figure 6b was symmetrical and showed an expected pKa value of 4.82 (typical for simple carboxylic acids), and there was no apparent insoluble material generated during the titration. In sharp contrast, the first derivative of the titration curve for the compound in Figure 6a was highly skewed and showed a minimum at the surprisingly high value of pH 5.8, with the approximate mid-point of the titration at pH 5.6. Further, there was massive precipitate formed with each addition of HCl, starting when the pH dropped below 5.8.

These dramatic differences in titration properties between compounds of nearly identical structure support the inference that the structure in Figure 6a is forming the predicted internal acid-specific H-bond, and that H-bond is acting to increase the pH of transition between the ionic and non-ionic forms. Conversely, while the structure in Figure 6b may be forming internal H-bonds, if so then because an H-bond can form with either the carboxylate salt form or the

carboxylic acid form, such H-bonds do not significantly favor the non-ionic form over the ionic form and so do not significantly alter the pH of the transition between the two forms, relative to what one would find for a simple carboxylic acid such as propionic acid.

Example 3. Effect of low-conformational-freedom H-bond on pH of transition between ionic and non-ionic forms

Titration in aqueous solution of the acyclic structure of Figure 7b showed an expected pH of transition value of 4.95 (typical of simple carboxylic acids), with no precipitation of the acid form. In contrast, titration of the 5-membered ring structure of Figure 7a showed an unexpectedly-high value of 5.6 for the pH of transition between anionic and non-ionic forms. Titration of that ring structure was also accompanied by massive precipitation when the pH dropped below about 5.8, and the shape of the titration curve was highly asymmetric.

However, when these two compounds were instead titrated in the semi-aqueous medium, methanol/water, 1:1 by volume, there was no sign of insolubility and the first derivative plots of the titration curves were now symmetrical. For the acyclic compound shown in Figure 7b the pH at the mid-point of the titration was 5.8, while the pH at the mid-point of the titration was a substantially higher 6.45 for the ring structure in Figure 7a.

Thus, even in the absence of skewing of the titration curve due to precipitation of the lipophilic acid form, in the semi-aqueous titration experiment one sees a substantial 0.65 pH unit increase in the mid-point of the titration curve for the cyclic compound in Figure 7a. The most reasonable explanation for this seems to be that in this semi-aqueous solution an internal acid-specific H-bond is forming only in the case of the cyclic ring structure, and that H-bond is driving the equilibrium in favor of the acid form.

Example 4. Importance of insulating carboxyl from electron withdrawing effects of linked groups

Molecular modeling of structure 8b suggests that an internal H-bond should be strongly favored by the near-perfect geometry of said H-bond and the very limited conformational freedom between the H-bond donor and the H-bond acceptor moieties. In spite of these factors favorable to formation of an internal

acid-specific H-bond, when the salt of the structure in Figure 8b was prepared and titrated with HCl in aqueous solution, the titration curve indicated a pKa value below 3.0.

It is inferred from this result that the inductive effects from the amide, where said effects should be readily propagated through the intervening double bond, probably cause a large reduction in the pKa of the carboxyl, with those negative inductive effects far surpass any positive effect an internal acid-specific H-bond might have on raising the pKa of the carboxyl.

Example 5. Advantage of partially shielding H-bonding site from aqueous solvent

The two compounds in Figure 9 were prepared and then titrated in the semi-aqueous solvent, methanol/water, 1:1 by volume. This semi-aqueous solvent was used in these titrations in order to prevent insolubility effects from impacting the titration results. In these experiments the first derivative of the titration plots were symmetrical and there was no visible sign of precipitation. Thus, the objective of avoiding insolubility effects was met.

What was found is that structure (a) of Figure 9 showed a mid-point for the titration curve at a pH of 5.6, while structure (b) of Figure 9 showed a significantly higher mid-point for the titration at pH 6.14.

Again, the likely explanation for the significantly higher pH of transition value for structure (b) in these titrations is that in this semi-aqueous solution structure (b) is forming an internal acid-specific H-bond that is acting to shift the equilibrium in favor of the acid form, while the closely related structure (a) fails to form such an internal acid-specific H-bond. It seems likely that the moderate shielding of the H-bonding site in structure (b) is the factor which favors formation of an H-bond in structure (b), while lack of such shielding of the H-bonding site in structure (a) is likely responsible for the apparent absence of an internal H-bond in structure (a).

Example 6. Preparation and titration of two pH-switch structures designed to form an internal acid-specific low-barrier H-bond

The methyl ester of Isonipocotic acid was reductively alkylated with 3-

Pentanone and then the resultant tertiary amine converted to the N-oxide with meta-Chlorobenzoylperoxide. Finally, the ester was cleaved with aqueous sodium hydroxide to give structure iii in Figure 10b. This product, in its sodium carboxylate form, was then titrated in water. The first derivative of its titration plot shows two minimums, one at pH 3.8 (which is approximately that expected for the N-oxide moiety), and another minimum at pH 6.0, which is substantially higher than would be expected for either the N-oxide moiety or the carboxylic acid moiety. However, in light of the results in the earlier examples herein, the most likely explanation is that this pKa 6.0 value is due to an internal low-barrier H-bond forming between the carboxyl H-bond donor and the N-oxide H-bond acceptor, and that internal H-bond acts to drive the equilibrium toward the acid form - ie., raise the pKa.

The cis(3-amine-1-acid) derivative of camphoric acid was reductively alkylated with an excess of acetaldehyde and the resulting tertiary amine product converted to the N-oxide with meta-Chlorobenzoylperoxide to give structure i in Figure 10b. This product, in its sodium carboxylate form, was then titrated in water. The first derivative of its titration plot shows two minimums, one at pH 4.2 (which is approximately that expected for the N-oxide moiety), and another minimum at pH 6.5, which is substantially higher than would be expected for either the N-oxide moiety or the carboxylic acid moiety. Again in light of the results from earlier examples herein, the most likely explanation for this exceptionally high pKa value of 6.5 is that an internal low-barrier H-bond is forming between the carboxyl H-bond donor and the N-oxide H-bond acceptor, and that internal H-bond acts to strongly drive the equilibrium toward the acid form, thereby raising the pKa value.

It is noteworthy that the above internally H-bonding isonipocotic acid derivative has an appreciably lower pKa value (6.0) than the somewhat similar internally H-bonding camphoric acid derivative - in spite of the H-bond donor moiety and the H-bond acceptor moiety being virtually identical in both structures. The most likely explanation for this significant difference between pKa values is that the lowest energy conformation for 6 membered aliphatic rings, such as the isonipocotic structure above, is typically the chair conformation and it commonly takes something like 5 Kcal to convert that chair conformation to the next most stable conformation, the twisted boat form. Because the inventor's molecular

modeling has shown that only the twisted boat conformation of the isonipocotic derivative can form the internal acid-specific H-bond, it is likely that the strength of that H-bond is attenuated by the energy cost of converting from the chair to the twisted boat conformation.

In contrast, the 5-membered aliphatic ring of the camphoric derivative can readily and at low energy cost shift between differing pucker conformations, and so there is little energy cost to adopting the conformation suitable for forming the internal acid-specific H-bond. As a consequence, essentially the full energy of that H-bond is available for favoring the acid form over the salt form - hence raising the pKa to the exceptionally high value of 6.5.

The significance of the above is that it demonstrates that subtle structural properties can be exploited for rather precisely adjusting the pKa value of a pH-switch in order to achieve a desirable balance between efficacy and specificity, the importance of which is illustrated in Figure 12.

Example 7. Titration procedures for studying pH-switch structures

a) Aqueous titrations

Typically new structures that may be suitable as advanced pH-switch components are first assessed for their pT value, that is, the pH value at the mid-point of their transition between anionic and non-ionic forms. This entails preparing the sodium salt form of the structure and dissolving it at a concentration of 5 milliMolar in 50 ml of 0.15 Molar NaCl (deareated to minimize carbonic acid content). A small magnetic stir bar is added and the pH of the solution adjusted to about 9 with a small amount of 1 M NaOH. While stirring, 5 microLiter portions of 1 M HCl are added and the pH is recorded at 1 minute after addition of each HCl portion. If precipitation or oiling out of the pH-switch has not occurred during the course of the titration, a plot of pH versus volume of added HCl affords the conventional titration curve. However, a far more useful presentation of the titration results, which allows a rather precise measure of the pKa value, is obtained by instead plotting the first derivative of this titration curve. This entails plotting on the X axis the values for: (pHn + pHn+1) / 2, and plotting on the Y axis the values for: (pHn - pHn+1).

b) Semi-aqueous titrations.

If the pH-switch precipitates or oils out during the course of the titration then the titration curve will generally be strongly skewed. In such cases it is difficult to differentiate between solubility effects and pKa effects on the mid-point of the transition between anionic and non-ionic forms of the pH-switch. In such cases the solubility effect can be avoided by titrating in a semi-aqueous solvent where both the salt and acid forms of the pH-switch are fully soluble. A 1:1 mix of methanol and water generally serves for this purpose. In this solvent the first derivative titration curves again become symmetric. In the case of a substance which cannot form a stable internal H-bond, such as octanoic acid, the titration plot now shows a classic pKa value very similar to that seen for a fully water soluble simple carboxylic acid, such as propionic acid. However, in the case of a substance designed to form a stable internal acid-specific H-bond, such as the advanced pH-switch structures described in the Examples above, while the titration plot does become symmetric in methanol/water, nonetheless, the titration plot can also show a substantially increased pKa value relative to a very similar structure which cannot form an internal acid-specific H-bond - and this increased pKa value is indicative of an internal acid-specific H-bond forming under the conditions of the titration.

Thus, in titrations in physiological saline the total increase in the apparent pH of transition, accompanied by asymmetry in the titration curve, can be due to a combination of an internal acid-specific H-bond effect plus a solubility effect. However, when the titration is instead carried out in a semi-aqueous solvent, such as methanol/water, a substantial rise in the pKa value for a substance designed to form an internal acid-specific H-bond, relative to the pKa for a very similar substance which cannot form such an H-bond, is principally attributable just to the internal H-bond, and not to any lipophilicity increase in the acid moiety which might be a consequence of that internal H-bond. However, it should also be appreciated that to some extent the lower-polarity methanol/water solvent inherently favors formation of an internal H-bond, and so when at all possible when testing a new pH-switch structural type it is desirable to confirm internal acid-specific H-bond formation by titrating in a fully aqueous solution.

Example 8. Cell culture test system for onco-tools

It is recommended that initially each prospective onco-tool structure should be tested in a relatively simple biological system wherein the onco-tool is exposed to the principal biological environments and structures it will encounter in a living subject - including particularly mammalian cells exposed to serum-containing medium buffered at pH 7.4 to emulate blood and normal tissues, and buffered at pH 6.4 to emulate acidic areas of tumors.

Such a suitable test system entails preparing two preparations of isotonic culture medium. One should contain 10% serum and be strongly buffered at pH 7.4 with 50 milliMolar HEPES buffer (pKa 7.5). The other should contain 10% serum albumin and be strongly buffered at pH 6.4 with 50 milliMolar BisTRIS buffer (pKa 6.5). Iodine-131 containing onco-tool should be added at equal concentration to each culture medium.

Before the experiment, Hela cells should first be grown to confluency in 12-well culture plates. Next, the culture medium is removed from four wells of cells and replaced with the onco-tool-containing medium buffered at pH 7.4. Further, culture medium is removed from another four wells of cells and replaced with the onco-tool medium buffered at pH 6.4. The plates are then incubated at 37 deg. C for one hour. After the incubation, the onco-tool-containing medium is removed and replaced with onco-tool-free medium of the same pH, swirled briefly, and removed. This wash procedure is repeated a total of 4 times. Next, the cells are lysed with 1 ml of detergent solution and that lysis solution removed and counted in a scintillation or gamma counter to provide a measure of the relative quantity of onco-tool which has been sequestered under each of the two pH conditions.

A preferred onco-tool structure is one which is maximally sequestered by the cells at pH 6.4, but only minimally sequestered by the cells at pH 7.4.

Example 9. Testing onco-tools in normal mice

The above cell culture test system for onco-tools allows an initial quick and quantitative assessment of the probable efficacy and specificity properties of a substantial number of prospective onco-tools. However, this initial assessment

should next be followed up for the most promising onco-tool structures with tests in living mice. It is recommended that the mice first be pre-treated with a carbonic anhydrase inhibitor, such as Acetazolamide, to assure that their urine remains basic for a number of hours. Next, a suitable quantity of Iodine-131-containing onco-tool in phosphate-buffered saline should be injected, preferably intravenous, such as into the tail vein. Thereafter the mice should be periodically monitored for a period of up to about 24 hours (such as by briefly positioning under a suitable gamma counter or gamma camera) to determine the rate of excretion of the labeled onco-tool.

The main purpose of this testing in normal mice is to identify any onco-tools which are unduly sequestered in normal tissues - presumably due to excessive lipophilicity, or possibly due to some structural element that has an unexpected affinity for normal tissues or some particular organ, etc. Onco-tools that are rapidly and thoroughly excreted from normal mice, and so pass this preliminary animal test, should next be tested in tumor-bearing mice, as described in the following Example.

Example 10. Testing onco-tools in tumor-bearing mice

While the above relatively simple tests in normal tumor-free mice allow one to discard those onco-tools which have an excessive affinity for normal tissues, the more decisive test for a prospective onco-tool structure is to test it in tumor-bearing mice.

In such experiments it is recommended that one first pre-treat the mice: a) with a carbonic anhydrase inhibitor, such as Acetazolamide, to raise the pH in the urine; and, b) with one or a combination of substances effective to selectively reduce the pH in tumors. Such substances include: i) glucose (Naeslund & Swenson (1953) Acta Obstet. Gyneocol. Scand. 32, 359 - 367); ii) the mitochondrial inhibitor, meta-iodobenzylguanidine (Jahde et. al., (1992) Cancer Research 52, 6209 - 6215); and, iii) vasodilator drugs routinely used to treat persons with hypertension (Adachi and Tannock (1999) Oncology Research 11, 179 - 185).

After an appropriate period of time following such pre-treatments, the tumor-bearing mice are injected (preferably intravenous) with the Iodine-131-containing onco-tool. Following a suitable period of time (on the order of 5 to 24

hours) to allow normal excretion by the kidneys of that portion of the administered dose which is not sequestered in tissues and/or tumors of the mice, the mice are killed and the major organs and any obvious tumors excised. Each organ and tumor and the remaining carcass is then counted in a gamma counter.

In regard to pre-treating the mice, it should be stressed that onco-tools will work best when both types of pre-treatments are used, where one type serves to prevent re-uptake of onco-tool into the cells lining the proximal tubules of the kidneys. This re-uptake is blocked by rendering the urine slightly basic. The other type serves to further increase the acidity (reduce the pH) in hypoxic/acidic areas of tumors. Preferably a combination of two or three such pre-treatments to selectively reduce pH in the tumors should be employed in order to adjust the tumor micro-environment so as to be best suited for effective and specific onco-tool activity.

The above presents a description of the best mode contemplated for the compositions and methods of the present invention, and of the manner and process of making and using such compositions and methods in such full, clear, concise, and exact terms as to enable any person skilled in the art to which they pertain to make and use the compositions and methods. These compositions and methods are, however, susceptible to modifications and alternate constructions from the illustrative embodiments discussed above which are fully equivalent. Consequently, it is not the intention to limit the disclosed compositions and methods to the particular embodiments disclosed. On the contrary, the intention is to cover all modifications and alternate constructions coming within the spirit and scope of the compositions and methods as generally expressed by the following claims, which particularly point out and distinctly claim the subject matter of the disclosed compositions and methods.

CLAIMS:

1. A composition containing an advanced pH-switch component, said advanced pH-switch component comprising:

a) an aliphatic ring structure selected from the group consisting of a 4-membered ring, a 5-membered ring, and a 6-membered ring;

b) a carboxylic acid moiety directly linked to said aliphatic ring structure, said carboxylic acid moiety is separated from any linked electron-withdrawing group by at least two carbons; and

c) an H-bond acceptor moiety selected from the group consisting of part of the aliphatic ring structure, directly linked to the aliphatic ring structure, and linked through one atom to the aliphatic ring structure;

wherein, said H-bond acceptor moiety in its non-ionic form has a structure which cannot serve as said H-bond donor moiety, and said carboxylic acid moiety and said H-bond acceptor moiety are positioned and oriented such that they are compatible with formation of an internal acid-specific H-bond.

2. The composition of Claim 1, wherein a stand-alone form of the H-bond acceptor moiety of the advanced pH-switch component has a pKa value in the range of approximately 3.0 to approximately 6.5.

3. The composition of Claim 1, wherein a H-bond acceptor moiety of the advanced pH-switch component is selected from the group consisting of:

(a) a cyanomethyl amine;

(b) a trifluoroethyl amine;

(c) an alkoxy amine;

(d) a hydrazine;

(e) an N-oxide;

(f) an imidazole;

(g) an aniline;

(h) an amide;

(i) a phosphoramide;

(j) a thiophosphoramide;

(k) a urea; and,

(l) an ether.

4. An onco-tool composition comprising:

(a) at least two pH-switch components, each pH-switch component being effective to undergo a pH-mediated transition between an anionic hydrophilic form at a higher pH and a non-ionic lipophilic form at a lower pH; and,

(b) at least one cargo component selected from the group consisting of:

i) a structure effective to bind a radioisotope, and

ii) a structure which contains a radioisotope.

5. The onco-tool composition of Claim 4, further comprising a radioisotope selected from the group consisting of:

(a) a radioisotope effective to report the presence of the composition;

(b) a radioisotope effective to kill cells; and,

(c) a radioisotope effective to report the presence of the composition and effective to kill cells.

6. The onco-tool composition of Claim 4, which has a specificity factor greater than 20.

7. The onco-tool composition of Claim 4, wherein carboxyl moieties of the pH-switches have pKa values in the range of 5.4 to 6.8.

8. The onco-tool composition of Claim 4, wherein two of the pH-switch components share a single H-bond acceptor moiety.

9. The onco-tool composition of Claim 8, wherein the shared H-bond acceptor moiety is an oxygen atom of an N-oxide.

10. An onco-tool composition comprising:

(a) at least two advanced pH-switch components, each comprising:

i) an aliphatic ring structure selected from the group consisting

of 4-membered ring, 5-membered ring, and 6-membered ring;

ii) a carboxylic acid moiety directly linked to the aliphatic ring structure, the carboxylic acid moiety being separated from any linked electron-withdrawing group by at least two carbons; and

iii) an H-bond acceptor moiety selected from the group consisting of part of the aliphatic ring structure, directly linked to the aliphatic ring structure, and linked through one atom to the aliphatic ring structure;

wherein the H-bond acceptor moiety in its non-ionic form has a structure which cannot serve as an H-bond donor moiety, and the carboxylic acid moiety and the H-bond acceptor moiety are positioned and oriented such that they are compatible with formation of an internal acid-specific H-bond; and,

(b) at least one cargo component selected from the group consisting of:

i) a structure effective to bind a radioisotope; and,

ii) a structure which contains a radioisotope.

11. A diagnostic onco-tool composition for detecting a tumor containing acidic areas, comprising:

(a) at least two pH-switch components effective to undergo a pH-mediated transition between an anionic hydrophilic form at a higher pH and a non-ionic lipophilic form at a lower pH; and,

(b) at least one radioisotope whose emission from within a tumor in the subject can be detected outside the subject.

12. The diagnostic onco-tool composition of Claim 11, wherein the radioisotope is a radiohalogen.

13. A therapeutic onco-tool composition for treating a subject having a tumor containing acidic areas, comprising:

(a) at least two pH-switch components effective to undergo a pH-mediated transition between an anionic hydrophilic form at a higher pH and a non-ionic lipophilic form at a lower pH; and,

(b) at least one radioisotope whose emission is effective to kill cells.

14. The therapeutic onco-tool composition of Claim 13, wherein the radioisotope is a radiohalogen.

15. The therapeutic onco-tool composition of Claim 13, wherein the radioisotope emits high linear energy transfer radiation.

16. The therapeutic onco-tool composition of Claim 15, wherein the radioisotope is selected from the group consisting of Astatine-211, Bromine-77, and Iodine-123.

17. The therapeutic onco-tool composition of Claim 13, wherein the radioisotope emits a beta particle.

18. A combination of therapeutic onco-tool compositions of Claim 13, comprising:

(a) at least one first onco-tool composition containing a first radioisotope which emits high linear energy transfer radiation; and,

(b) at least one second onco-tool composition containing a second radioisotope which emits a beta particle.

19. A diagnostic method for detecting in a subject a tumor containing acidic areas, the method comprising the steps of:

(a) providing a diagnostic onco-tool composition of Claim 11;

(b) introducing the diagnostic onco-tool composition into the subject;

(c) waiting for approximately 10 minutes to approximately 48 hours; and,

(d) scanning the subject with equipment effective to detect emissions from the diagnostic onco-tool composition in the subject.

20. The diagnostic method of Claim 19, further comprising the step of:

pre-treating the subject with at least one substance selected from the group consisting of:

(a) a first substance effective to increase the pH of the subject's urine,

and

(b) a second substance effective to decrease the pH in tumors.

21. A therapeutic method for treating tumors in a subject, the method comprising the steps of:

(a) providing a therapeutic onco-tool composition of Claim 13; and,

(b) introducing the therapeutic onco-tool composition into the subject.

22. The therapeutic method of Claim 21, further comprising the step of:

pre-treating the subject with at least one substance selected from the group consisting of:

(a) a first substance effective to increase the pH of the subject's urine, and

(b) a second substance effective to decrease the pH in tumors.

23. The therapeutic method of Claim 21, further comprising the step of:

irrigating the bladder of the subject for at least one hour following introducing the therapeutic onco-tool composition into the subject.

24. A therapeutic method for treating tumors in a subject, the method comprising the steps of:

(a) providing a combination of the therapeutic compositions of Claim 18; and,

(b) introducing the therapeutic compositions into the subject.

25. The therapeutic method of Claim 24, further comprising the step of:

pre-treating the subject with at least one substance selected from the group consisting of:

(a) a first substance effective to increase the pH of the subject's urine; and

(b) a second substance effective to decrease the pH in tumors.

26. The therapeutic method of Claim 24, further comprising the step of:

irrigating the bladder of the subject for at least one hour following the

introducing of the therapeutic composition into the subject.

27. A method for detecting in a subject a tumor containing an acidic area, and treating any of the tumors detected, comprising the steps of:

(a) providing a diagnostic composition effective to be sequestered in the acidic area of the tumor and containing a radioisotope which emits a signal that can be detected from outside the subject;

(b) introducing the diagnostic composition into the subject;

(c) waiting approximately 10 minutes to approximately 48 hours;

(d) scanning the subject with equipment effective to detect the signal emitted from the diagnostic composition; and, for the subject where the scan indicates the presence of the tumor;

(e) providing at least one therapeutic onco-tool composition effective to be selectively sequestered in the acidic areas of the tumor and containing a radioisotope effective to kill cells; and

(f) introducing at least one of the therapeutic onco-tool compositions into the subject.

28. The method of Claim 27, further comprising the step of:

pre-treating the subject with at least one substance selected from the group consisting of:

(a) a first substance effective to increase the pH of the subject's urine; and

(b) a second substance effective to decrease the pH in tumors,

wherein, the pre-treating step is performed before the introducing of the diagnostic composition into the subject and again before the introducing of the therapeutic onco-tool compositions into the subject.

29. The method of Claim 27, further comprising the step of:

irrigating the bladder of the subject for at least one hour after introducing the therapeutic onco-tool composition into the subject.

30. The method of Claim 27, further comprising the steps of:

repeating steps (a) through (d) after a period of time estimated to be sufficient for micro-metasteses to grow into a new tumor of sufficient size to contain acidic areas; and

repeating steps (e) and (f) if at least one tumor is detected in step (d).

31. A method for detecting and treating in a subject a tumor containing an acidic area, comprising the steps of:

(a) introducing into the subject a first substance effective to increase the pH of the urine in the subject;

(b) introducing into the subject a second substance effective to decrease the pH in the tumor;

(c) providing a diagnostic composition effective to be selectively sequestered in the acidic area of the tumor and containing a radioisotope which emits a signal that can be detected from the exterior of the subject;

(d) introducing the diagnostic composition into the subject;

(e) scanning the subject with a device effective to detect the signal emitted from the diagnostic composition;

(f) providing at least one therapeutic composition effective to be selectively sequestered in the acidic areas of the tumor and containing a radioisotope effective to kill cells; and

(g) introducing at least one of the therapeutic compositions into the subject.

32. The method of Claim 31, wherein the diagnostic composition is an onco-tool.

33. The method of Claim 31, wherein the therapeutic composition is an onco-tool.

34. The method of Claim 31, wherein the diagnostic composition and the therapeutic composition are an onco-tool.

35. The method of Claim 31, further comprising the steps of:

(a) waiting for the diagnostic composition to be sequestered in the acidic area of the tumor after introduction of the diagnostic composition into the subject;

(b) introducing into the subject an additional quantity of the first substance effective to increase the pH of the urine in the subject; and

(c) introducing into the subject an additional quantity of the second substance effective to decrease the pH in the tumor.

Figure 1. Chlorambucil

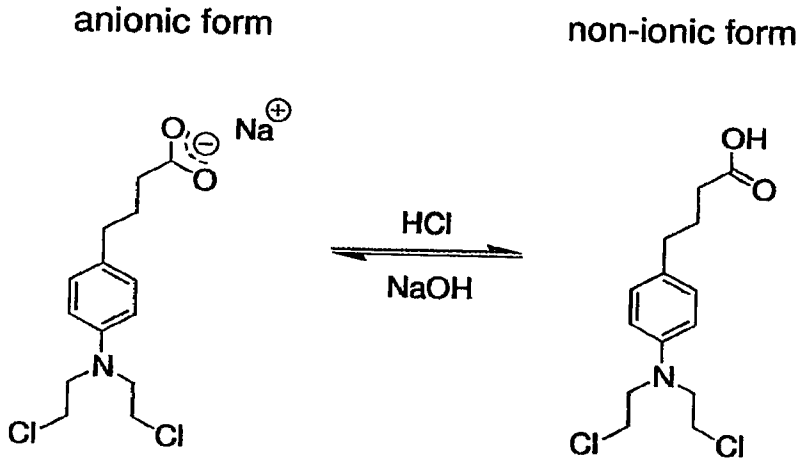


Figure 2. Distribution of Acidity in Tumors

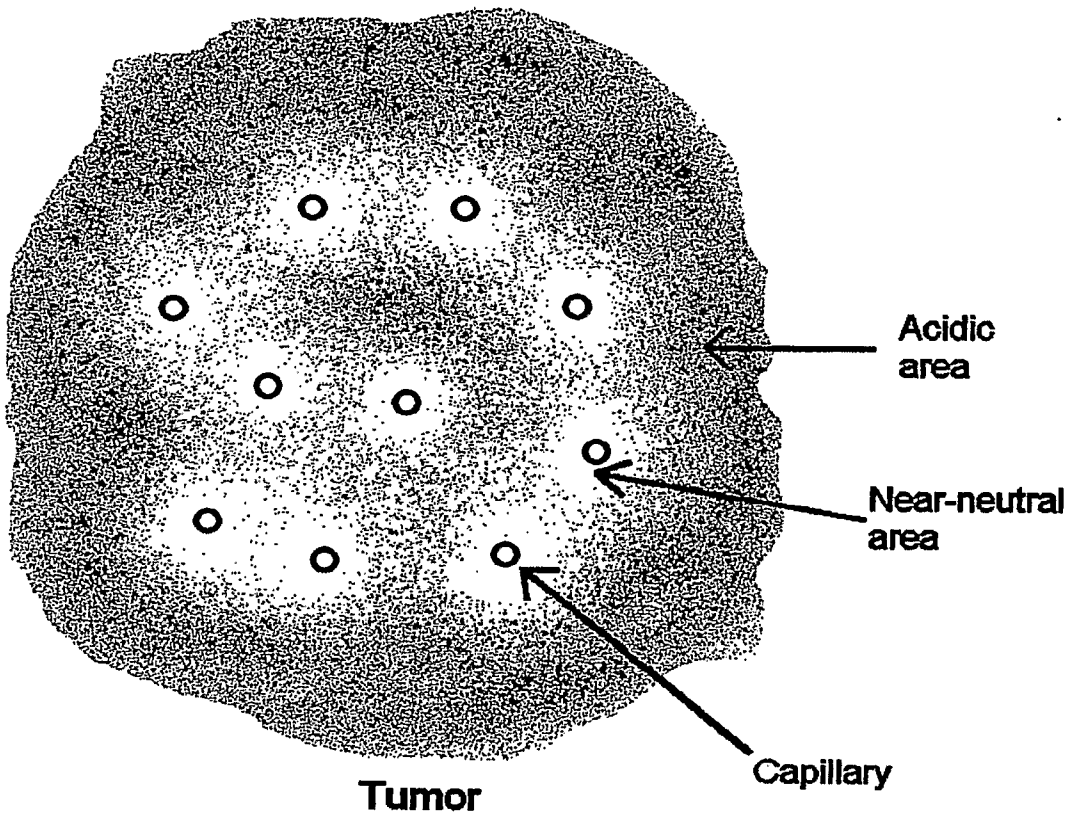
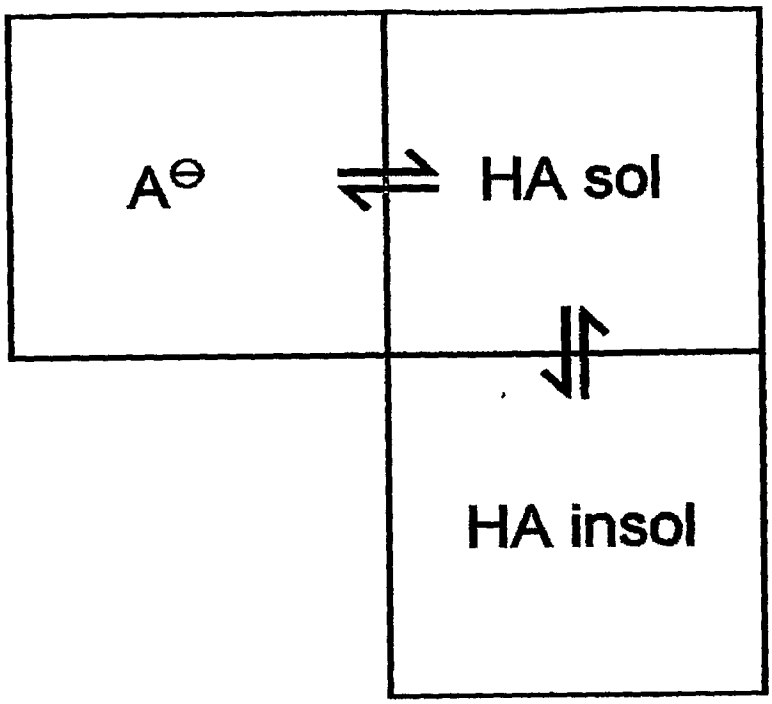


Figure 3. pH-Mediated Transition Between Forms



$$pH = pKa + \log \frac{[A^{\ominus}]}{[HA\ sol]}$$

$$[A^{\ominus}] + [HA\ sol] + [HA\ insol] = 1$$

$$P = \frac{[HA\ insol]}{[HA\ sol]}$$

P is octanol/water partitioning coefficient

Figure 4. Calculated Titrations as Function of Lipophilicity of Acid Form

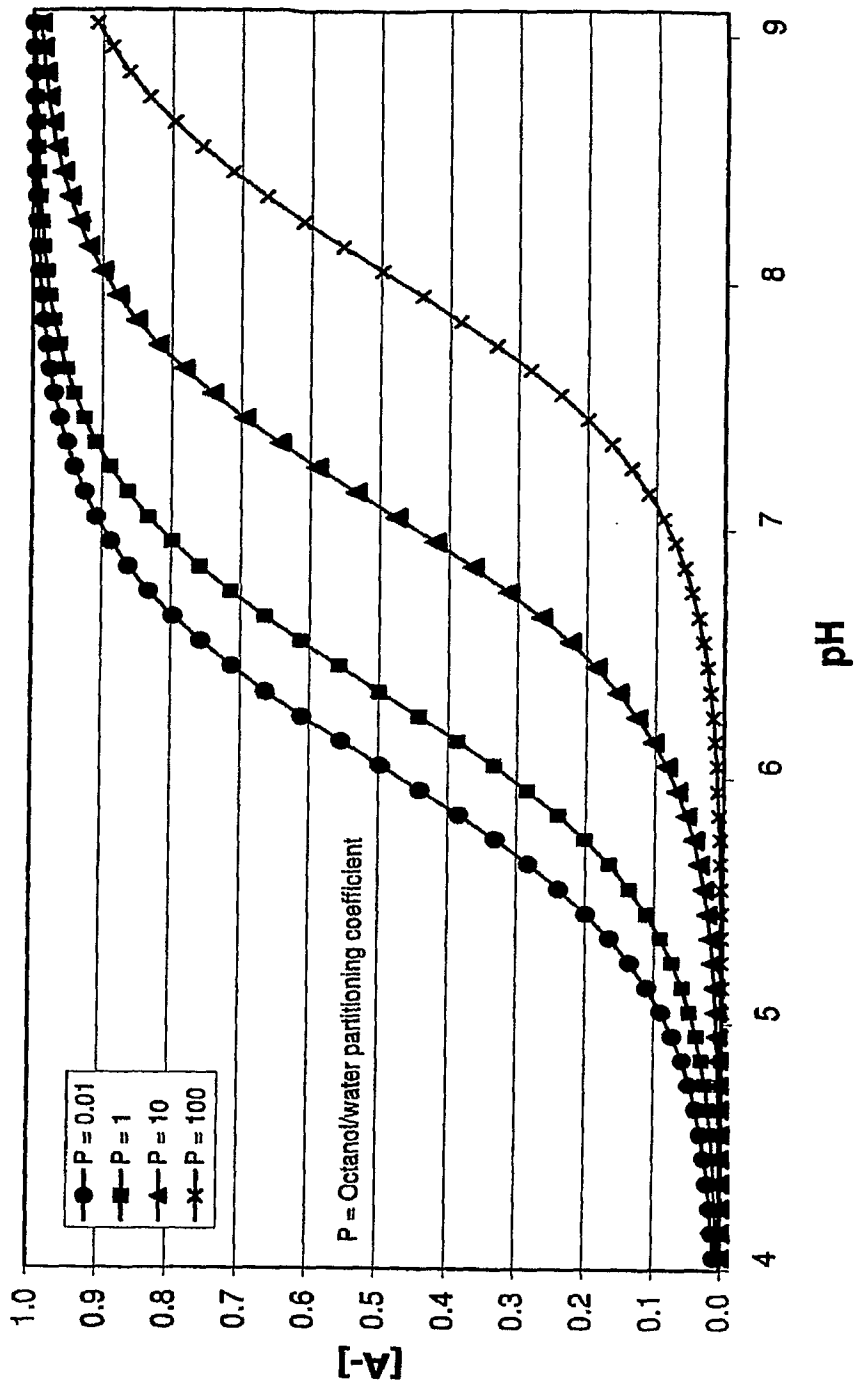
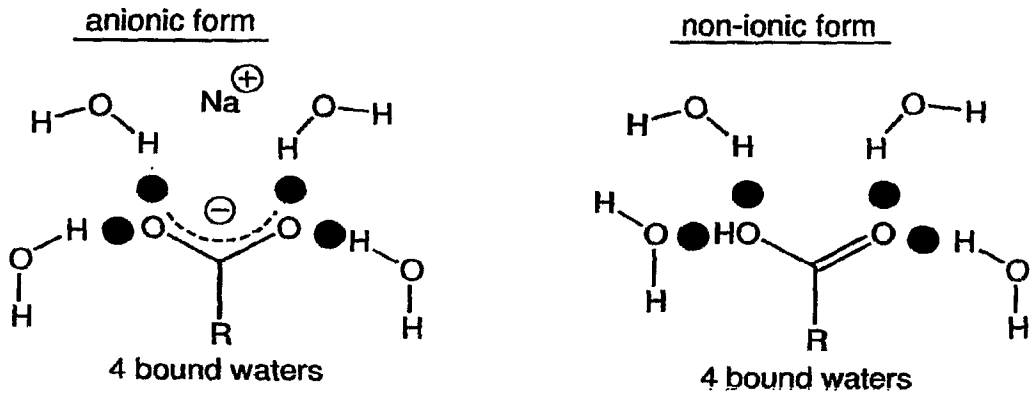


Figure 5. Expected Waters of Hydration

a) Conventional carboxylic acid



b) Internally H-bonded carboxylic acid

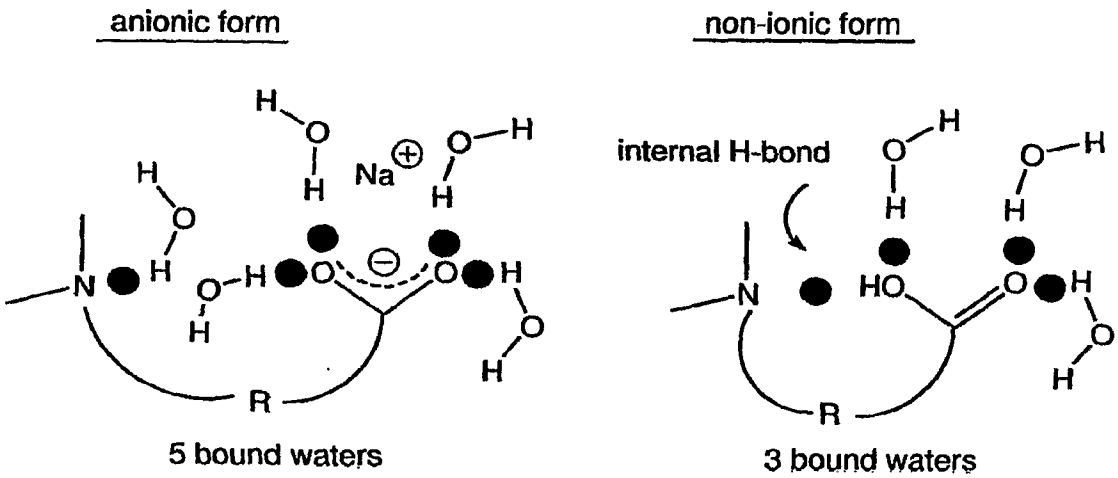
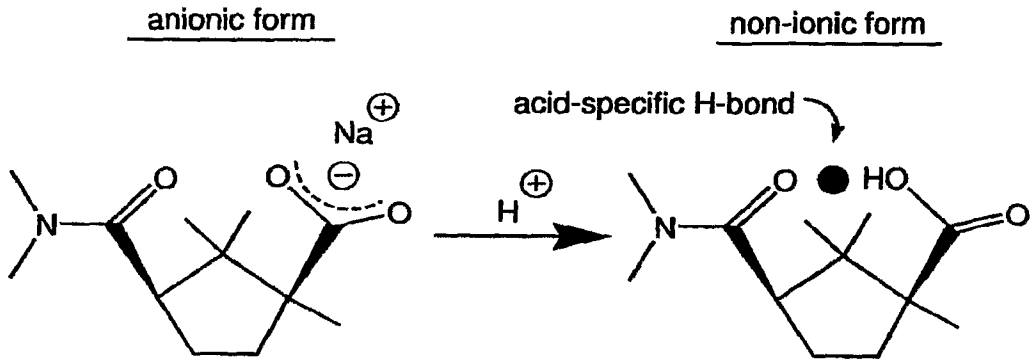


Figure 6. Acid-Specific H-Bond

a) Structure which forms acid-specific H-Bond



b) Structure which forms non-acid-specific H-bonds

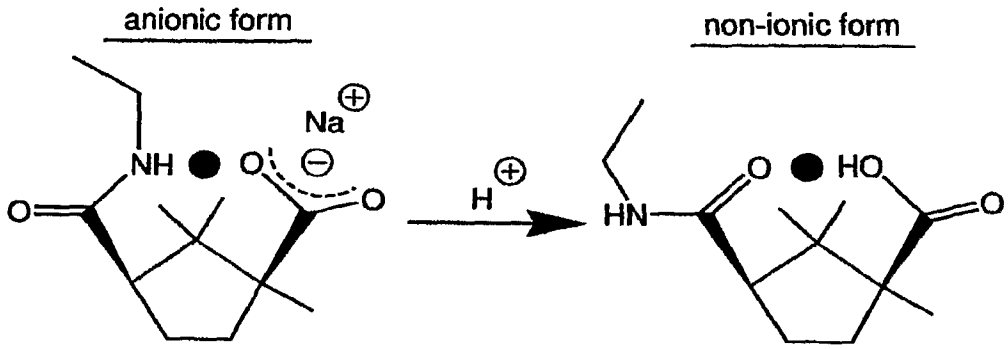
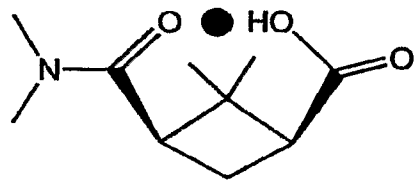


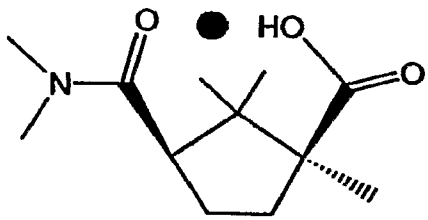
Figure 7. Representative Ring Structures Suitable for Advanced pH-Switches

a)

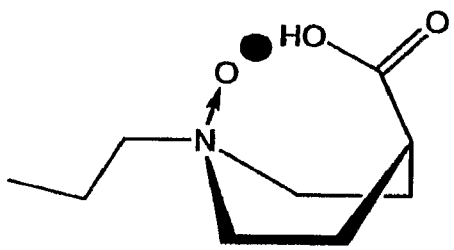
4-membered ring



5-membered ring



6-membered ring



b)

unacceptable structure

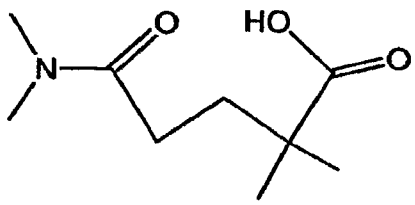
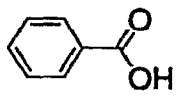
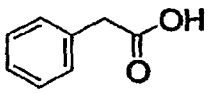
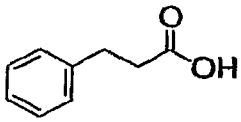
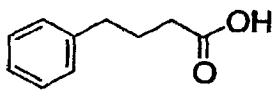
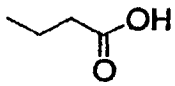


Figure 8. Insulation of Carboxyl from Inductive Effects

a) increasing insulation from inductive effects

Structure	# of carbons separating carboxyl and phenyl ring	pKa
	0	4.20
	1	4.31
	2	4.66
	3	4.75
	no phenyl ring	4.87

b) inadequate Insulation of Carboxyl from Inductive Effects

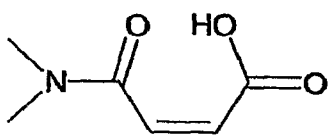
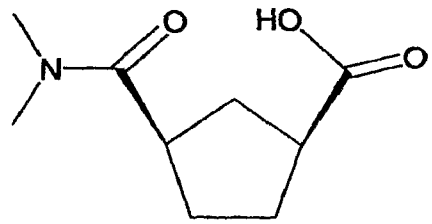
	pKa
	~2.0

Figure 9. Partial Shielding of H-bond Site

a) H-bond site open to solvent



b) H-bond site partially shielded from solvent

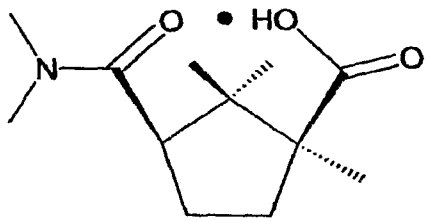
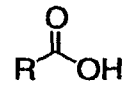
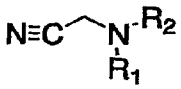
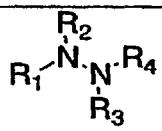
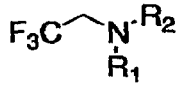
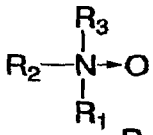
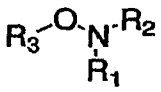
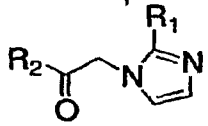
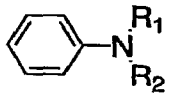


Figure 10. Low-Barrier H-bonds

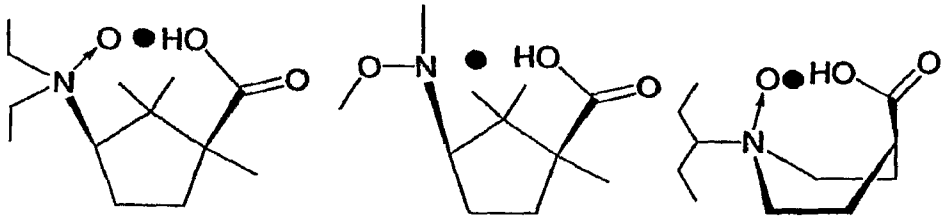
a) H-bond donor

Donor	approximate pKa
	5.0

b) Representative H-bond acceptors

Acceptor	approximate pKa	Acceptor	approximate pKa
	4.60		6.3
	4.75		4.6
	3.65		5.5
			5.0

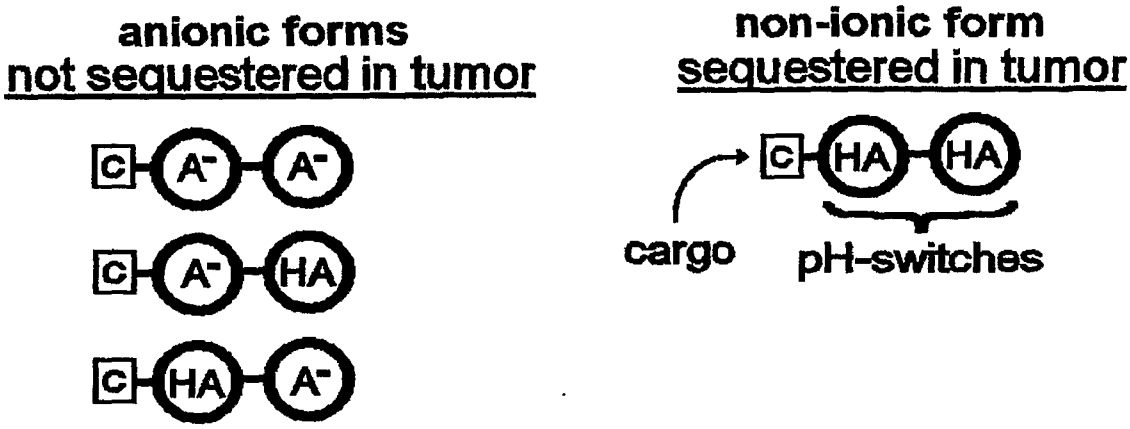
c) Representative pH-switches designed to form low-barrier H-bonds



i ii iii

Figure 11. Statistical Basis for Increased Specificity of Multi-pH-Switch Onco-Tools

a) OncoTool with two pH-switches



b) OncoTool with three pH-switches

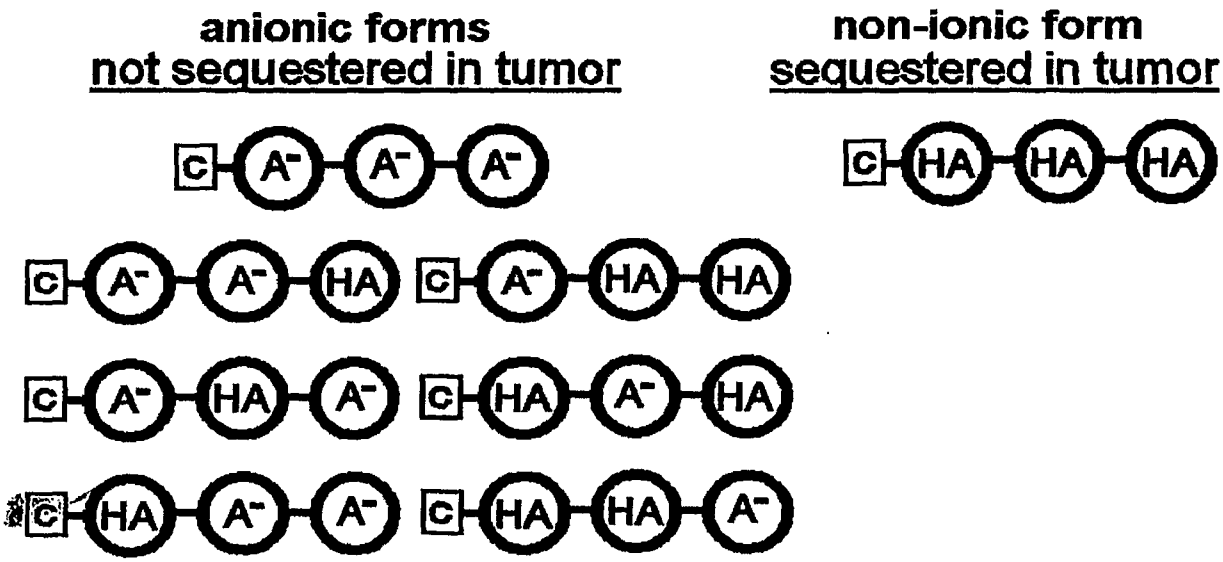


Figure 12. Calculated Efficacy and Specificity Factors

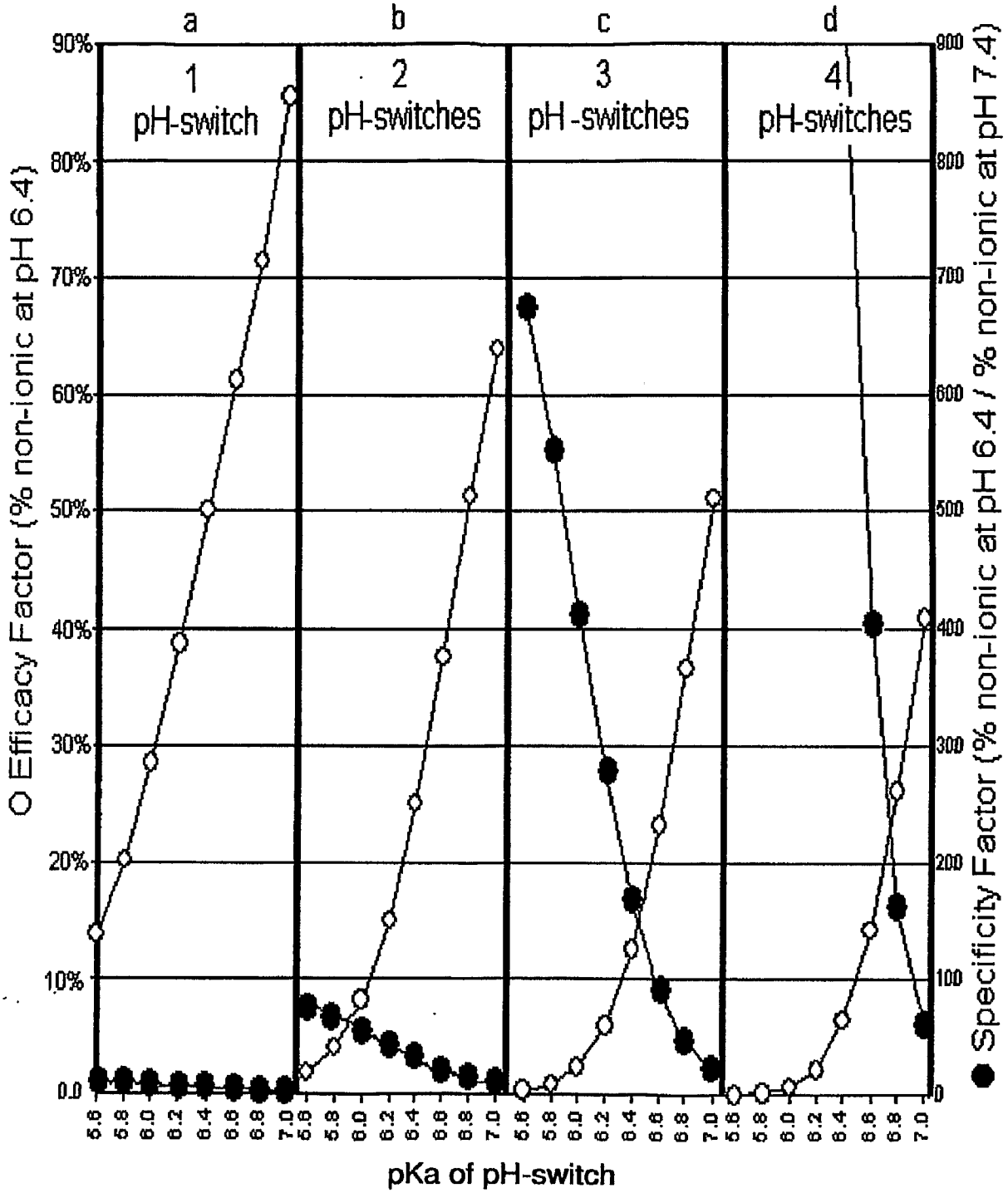
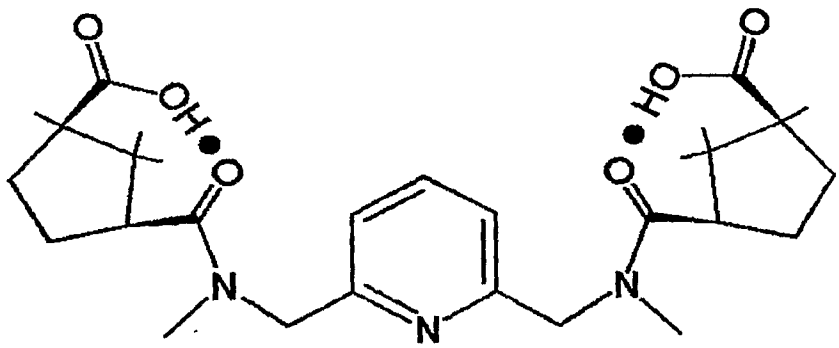


Figure 13. Composition Containing 2 pH-switches

a) structure



b) *n*-octanol/buffer partitioning

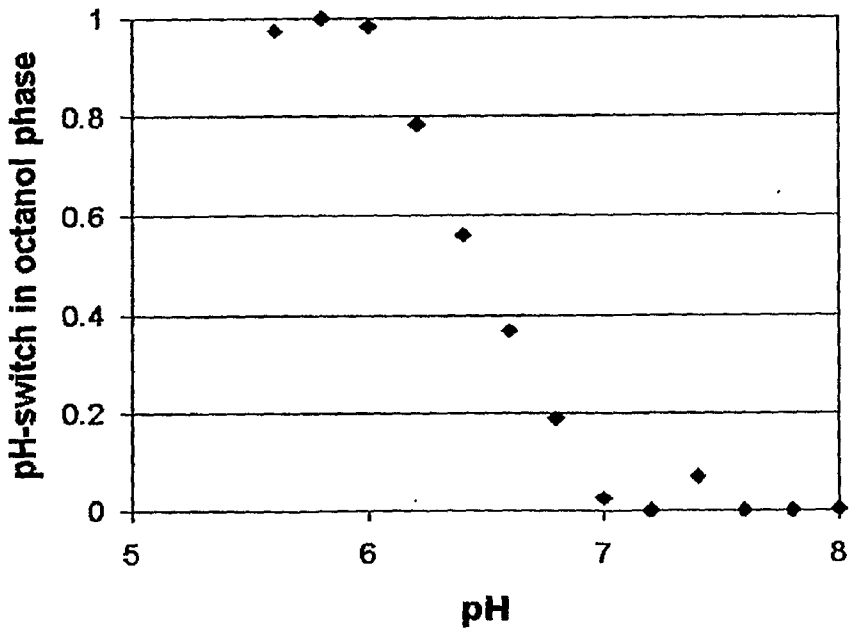
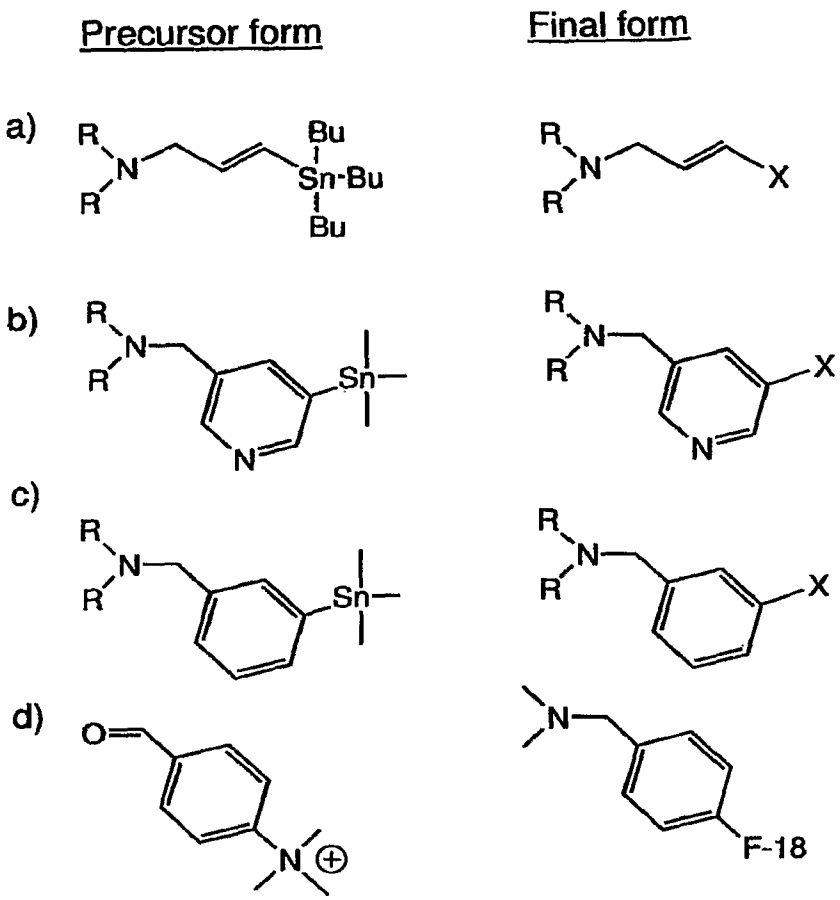


Figure 14. Representative Cargo Components



X = Br, I, or At radiohalogen

Figure 15. Synthetic Routes for Representative Cargo Components

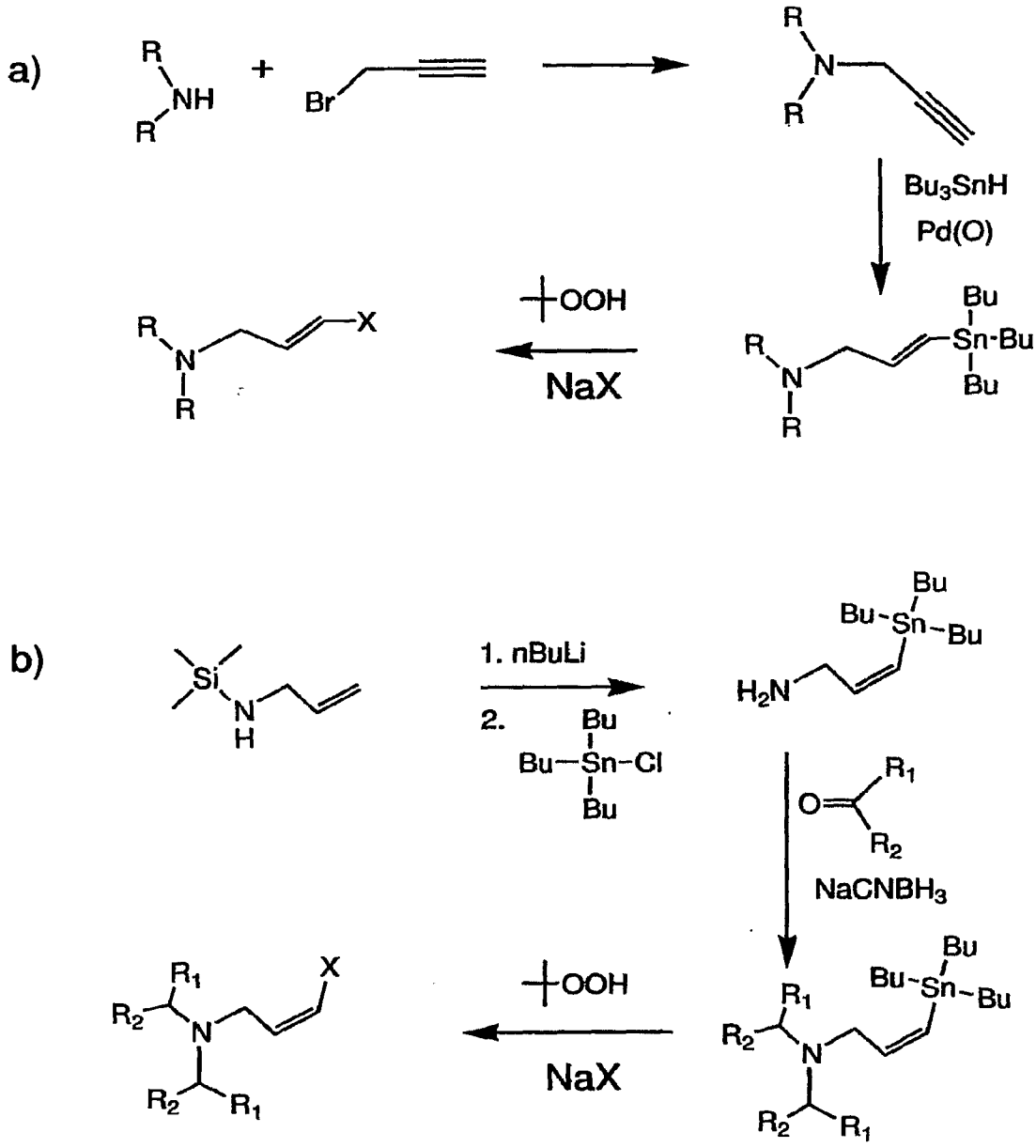
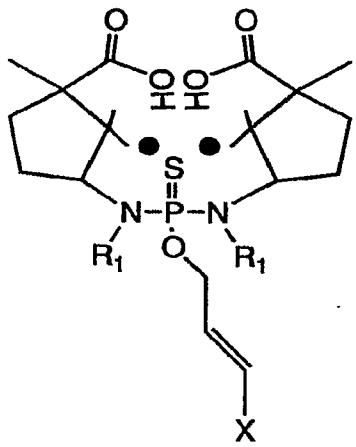


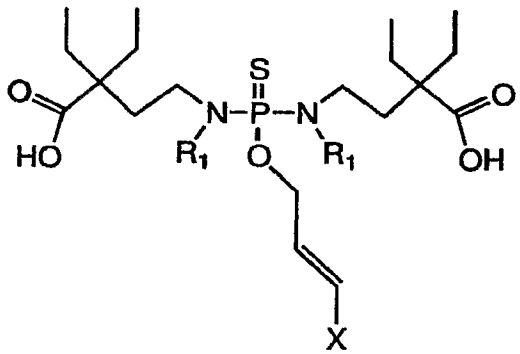
Figure 16. Representative Two-pH-Switch Onco-Tools

16a. Containing simple pH-switches

1.



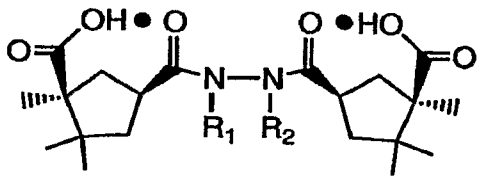
2.



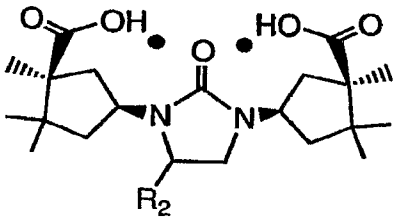
R_1 = alkane for adjusting lipophilicity
X = radiohalogen selected from F, Br, I and At

16b. Containing advanced pH-switches

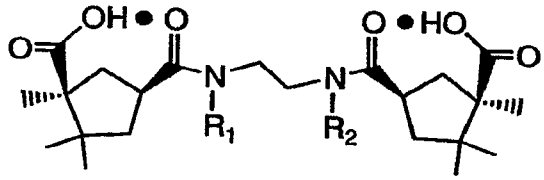
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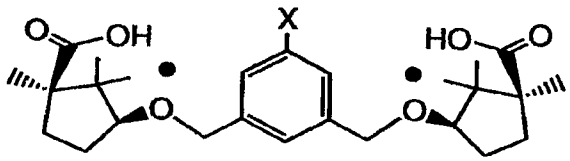
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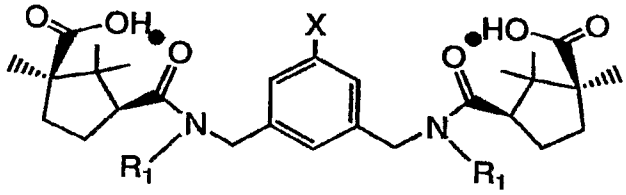
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4.



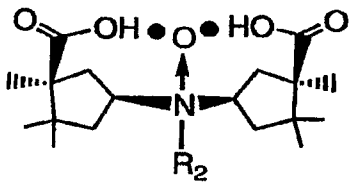
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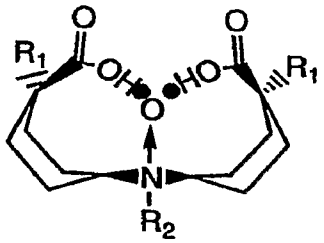
R_1 = alkane for adjusting lipophilicity
R_2 = cargo component containing radiohalogen (Fig. 14)
X = radiohalogen selected from F, Br, I and At

16c. Containing advanced pH-switches designed to form low-barrier H-bonds

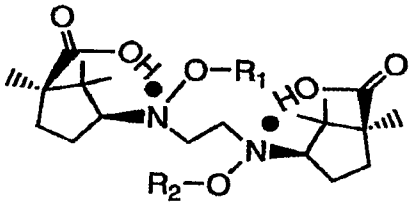
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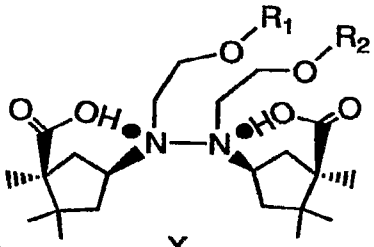
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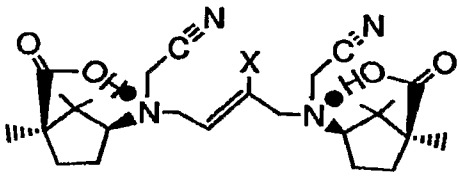
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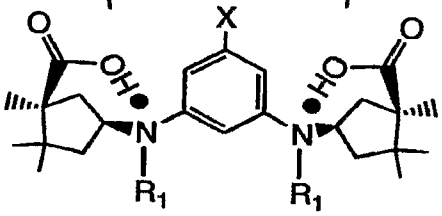
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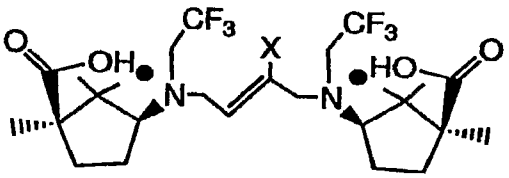
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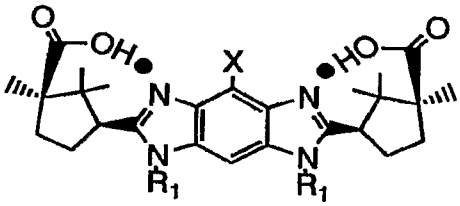
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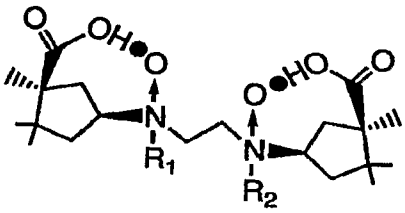
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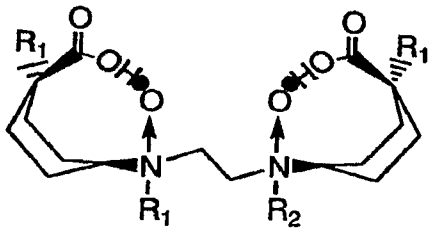
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9.



10.

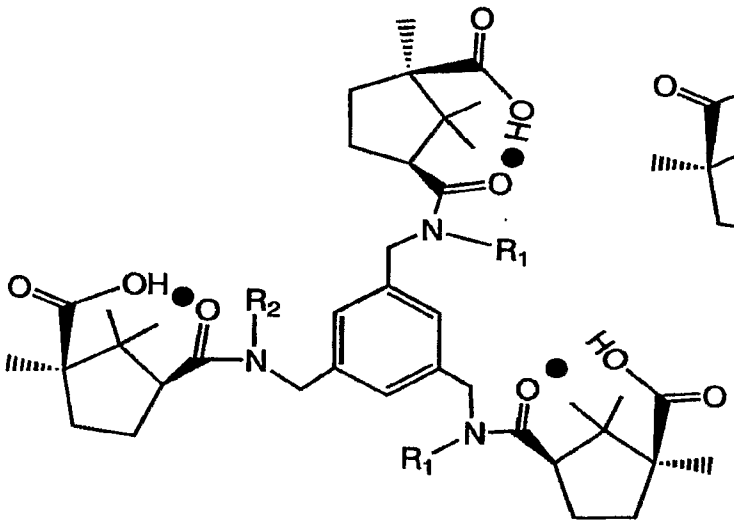


R_1 = alkane for adjusting lipophilicity
R_2 = cargo component containing radiohalogen (Fig. 14)
X = radiohalogen selected from F, Br, I and At

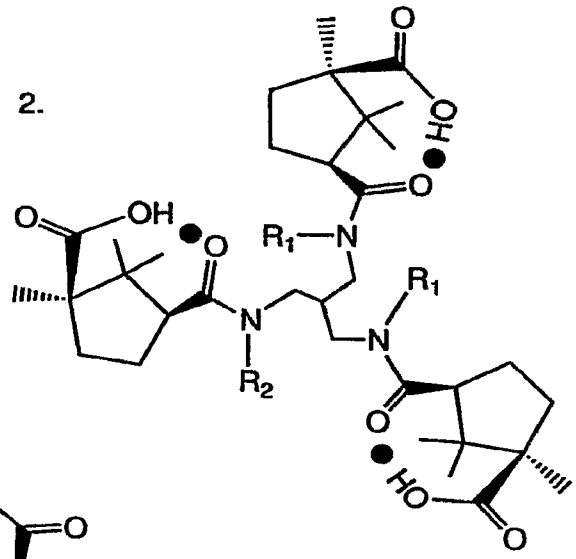
Figure 17. Representative Three-pH-Switch Onco-Tools

17a. Containing advanced pH-switches

1.

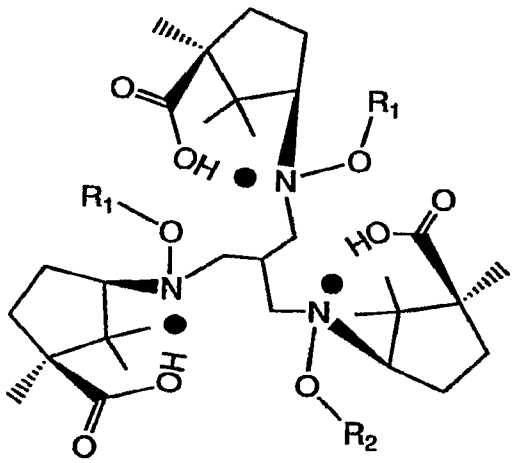


2.



17b. Containing advanced pH-switches designed to form low-barrier H-bonds

3.



4.

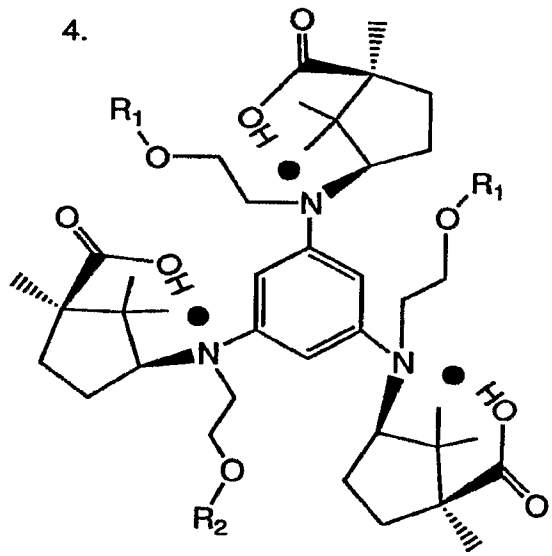
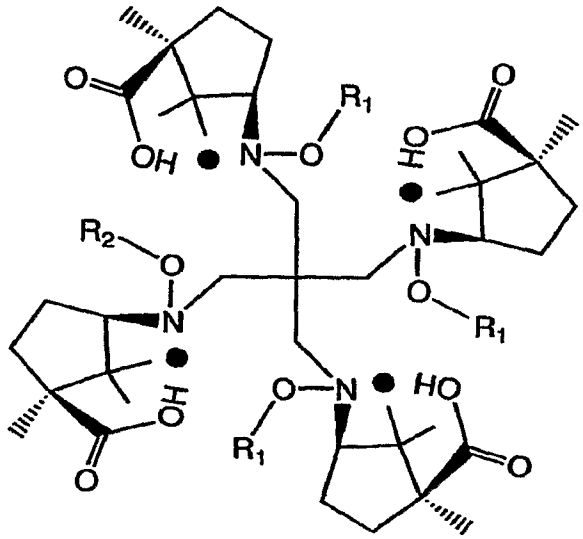


Figure 18. Representative Four-pH Switch Onco-Tools

a)



b)

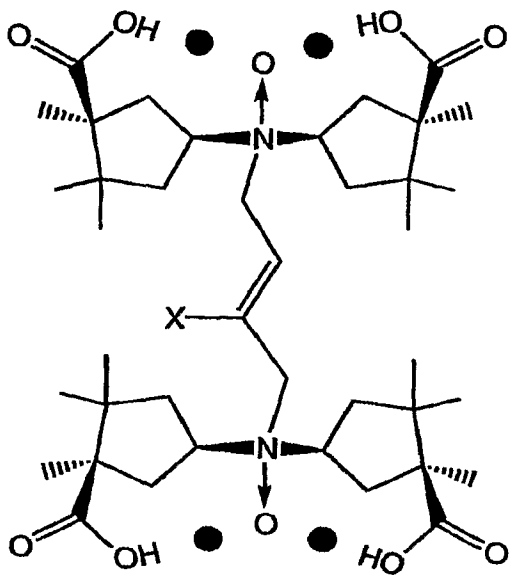


Figure 19. Synthesis of Onco-Tools Containing Simple pH-Switches

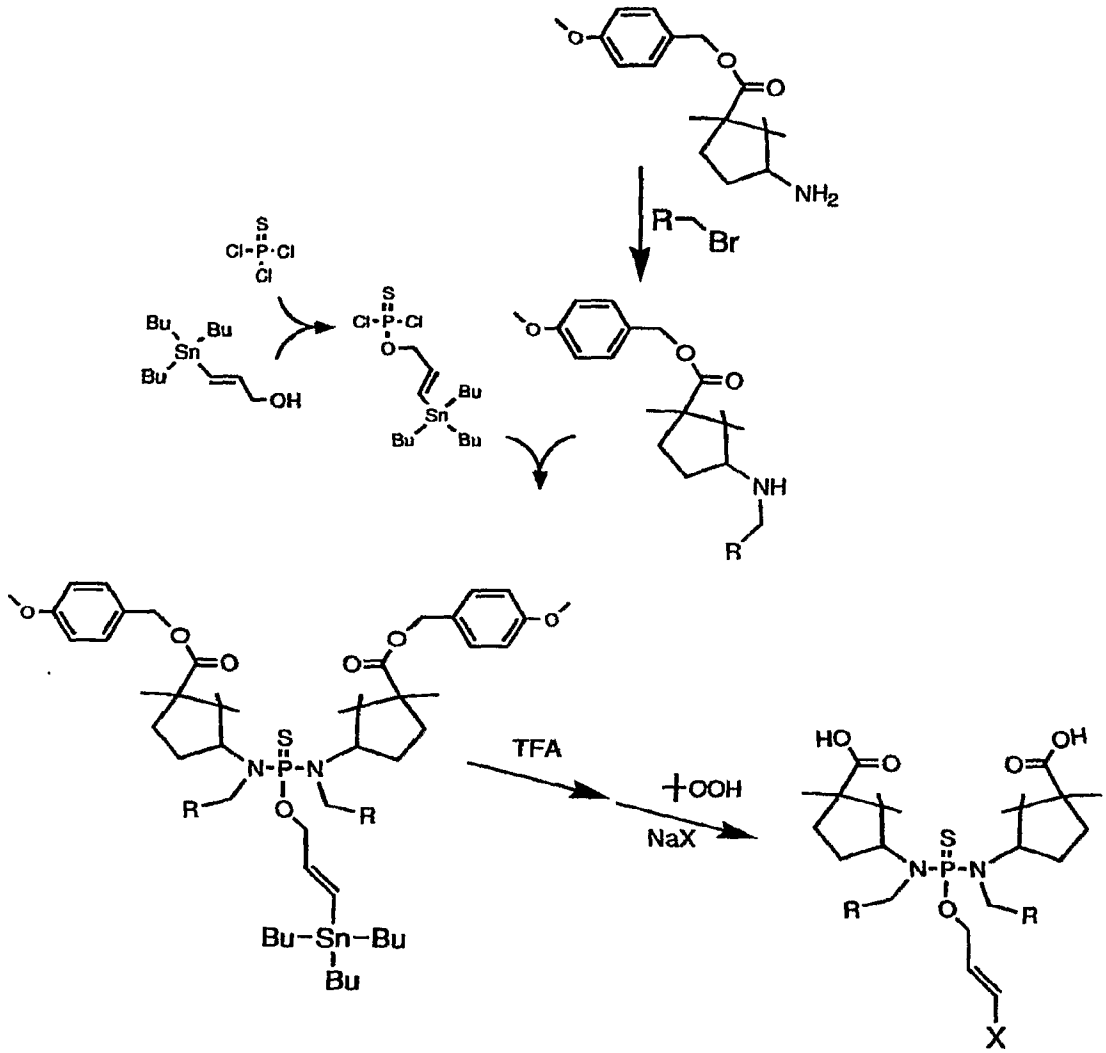
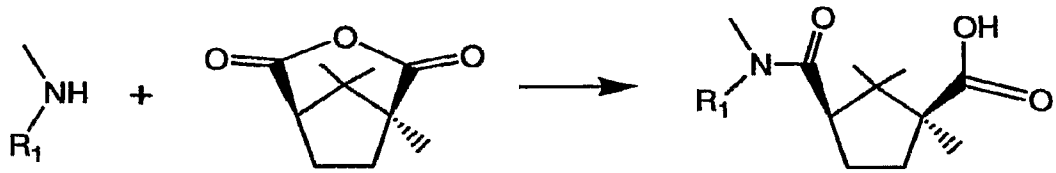


Figure 20. Synthetic Scheme for Advanced pH -Switches



R_1 = methyl, ethyl, propyl, butyl

Figure 21. Useful Intermediates for Onco-Tool Synthesis

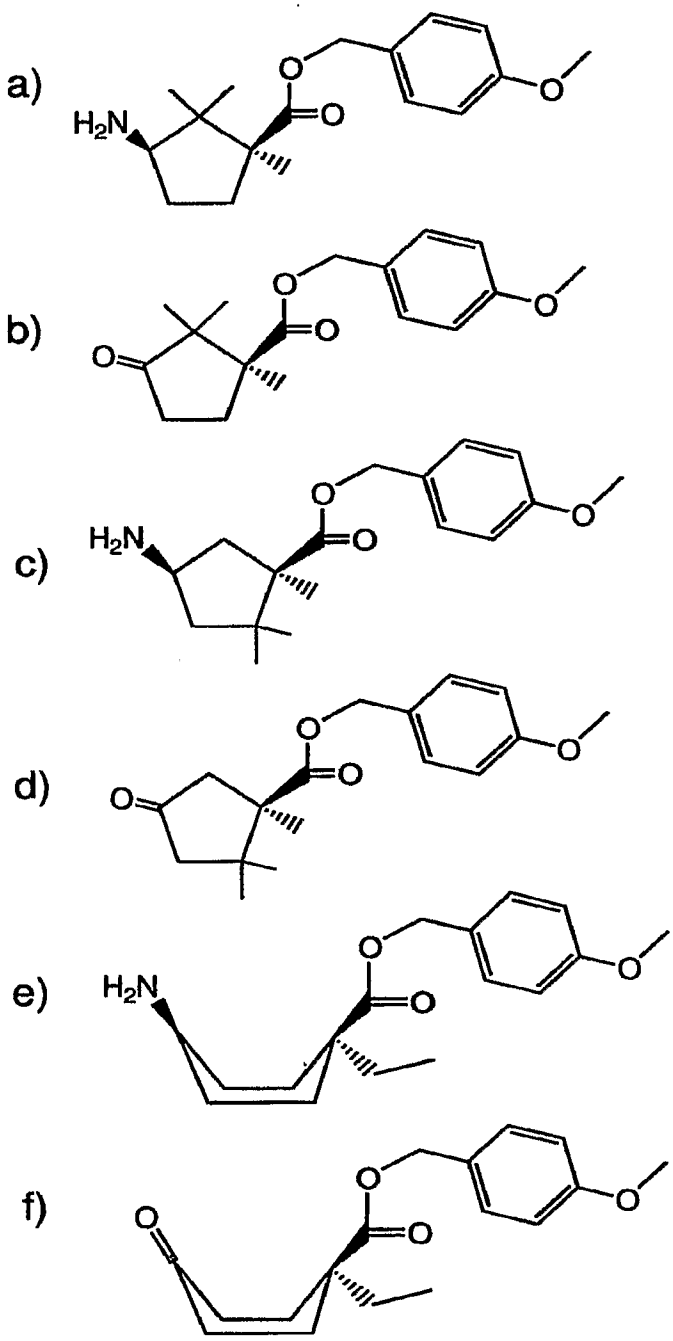


Figure 22. Representative Syntheses of pH-Switches

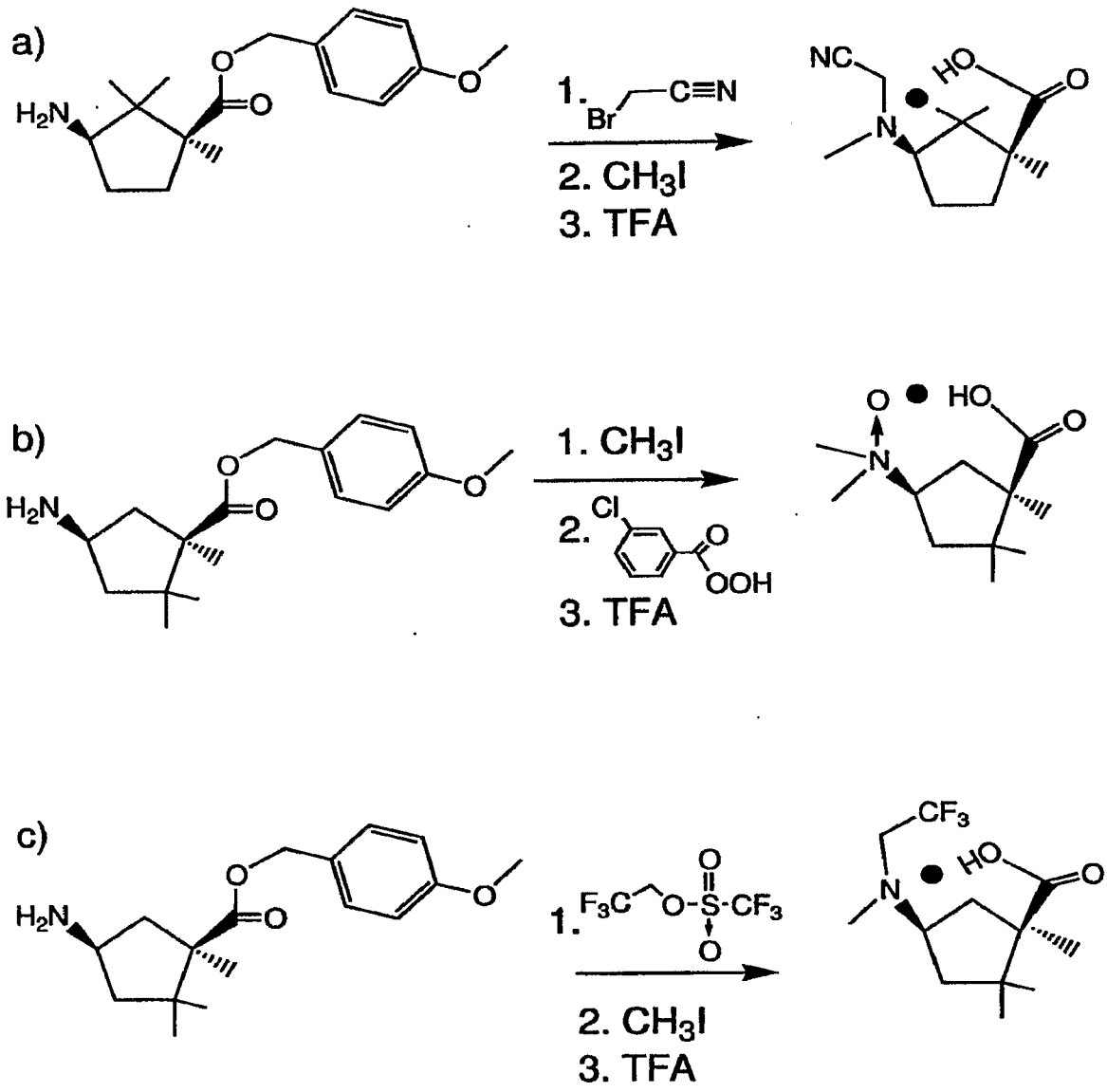
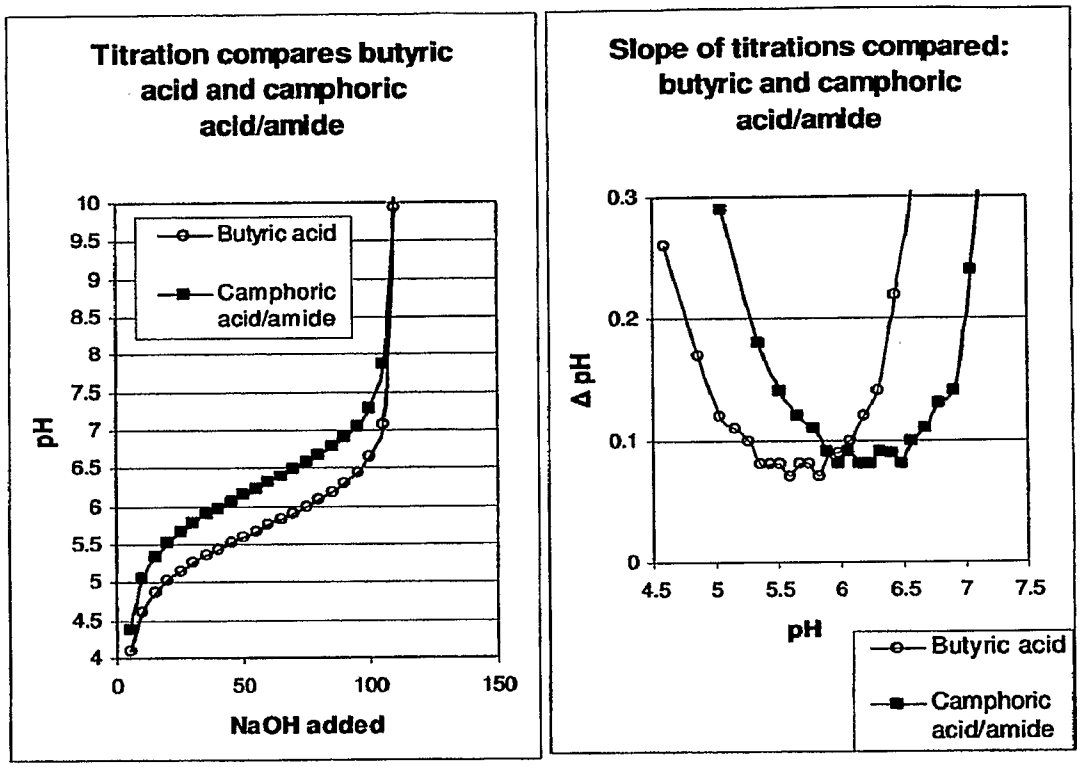
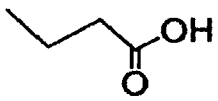


Figure 23. Titration Results

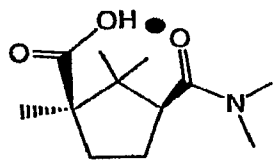
a) Simple carboxylic acid and advanced pH switch



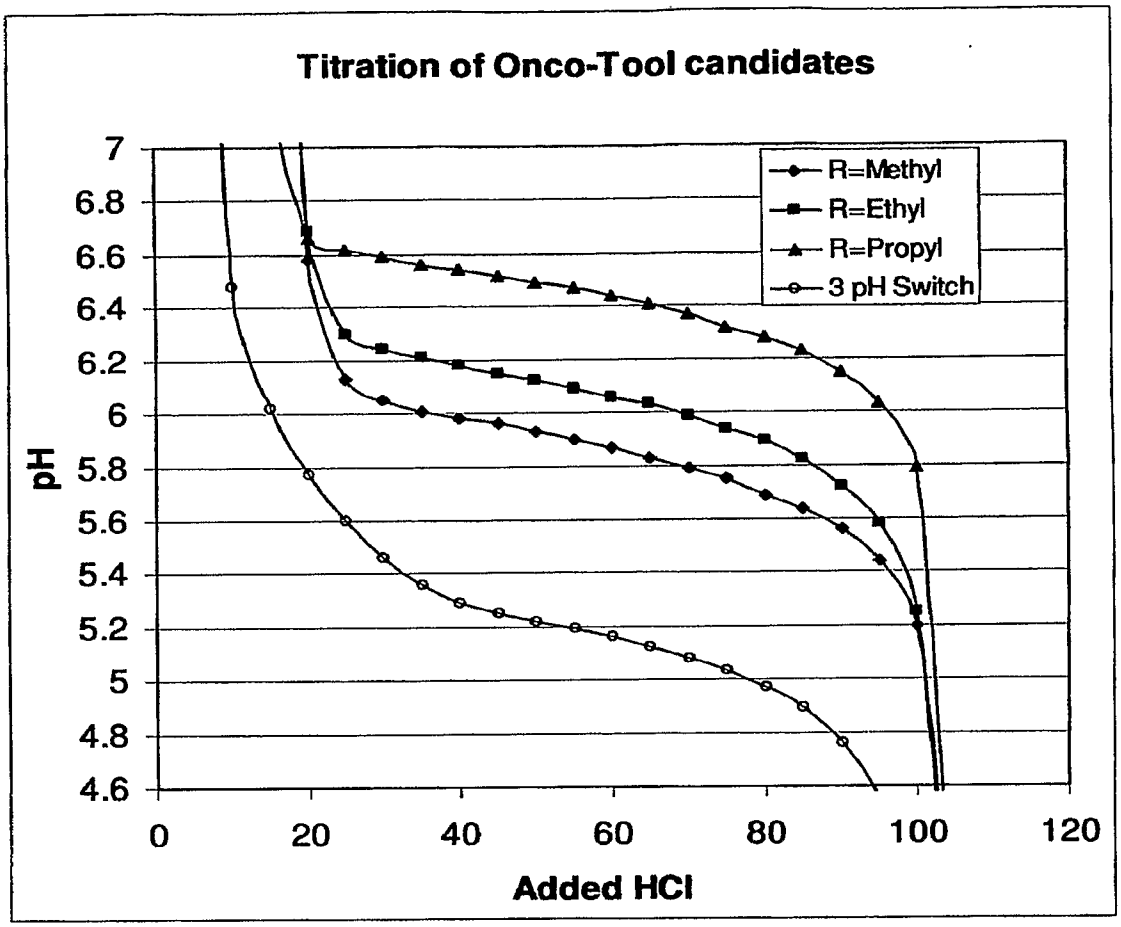
Butyric acid



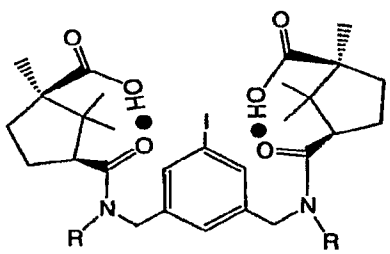
Camphoric acid/amide



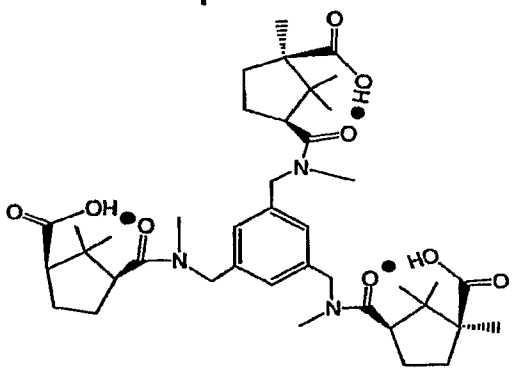
b) 2-pH-switch and 3-pH-switch structures



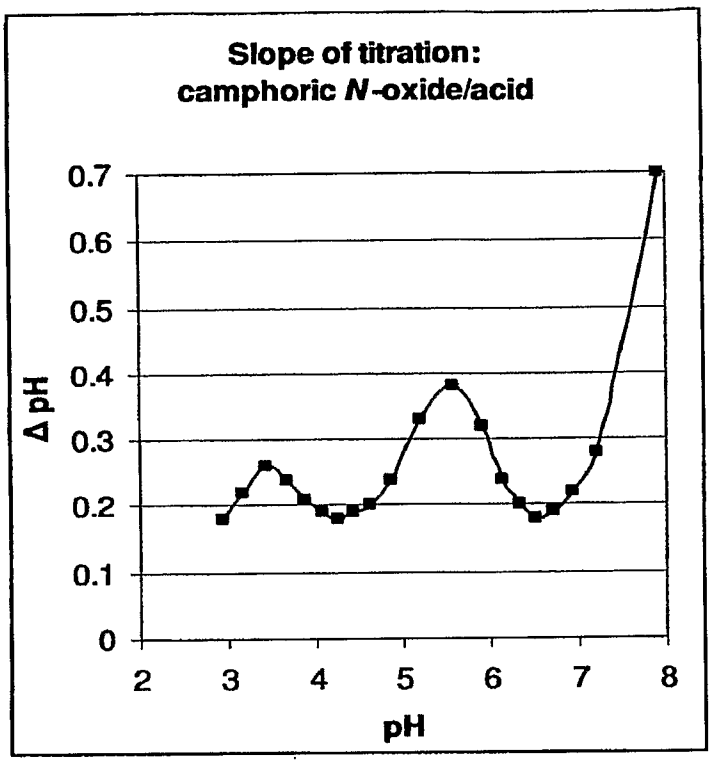
2-pH-switch



3-pH-switch



c) Advanced pH-switch designed to form low-barrier H-bond



Camphoric *N*-oxide/acid

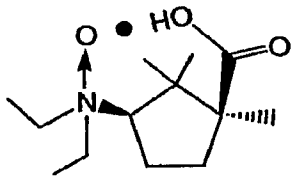


Figure 24. Experimentally-Determined pKa Values

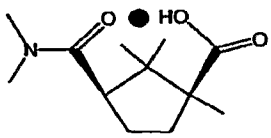
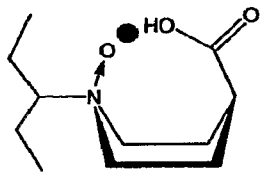
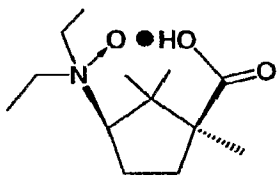
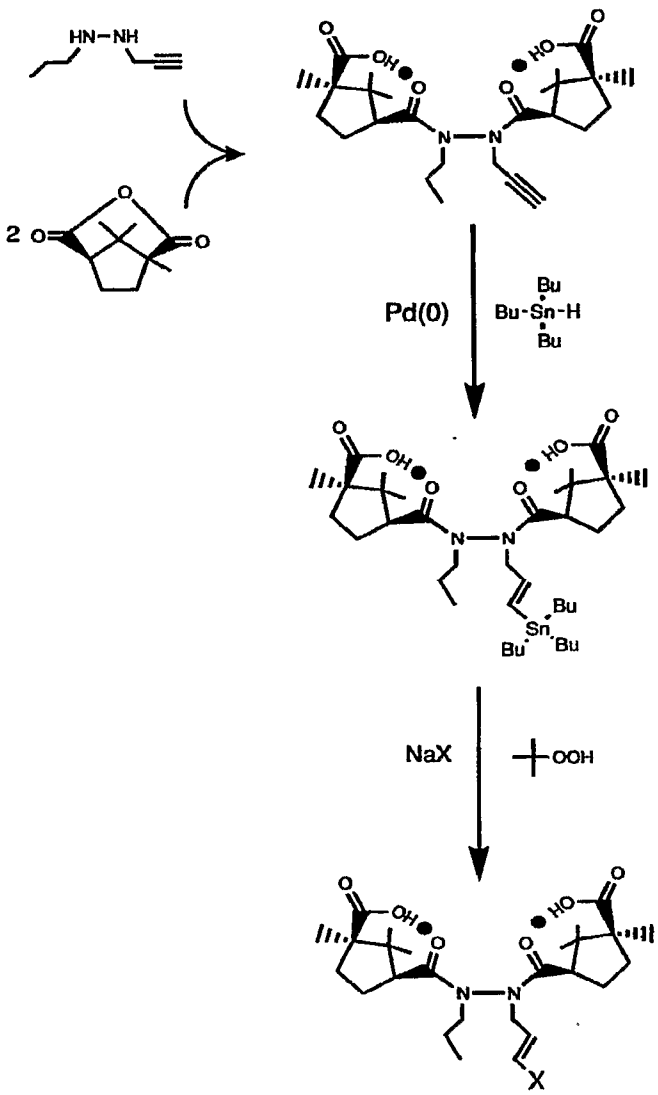
pH-switch structure	pH-switch type	Increase in pKa relative to propionic acid	Solvent for titration
	advanced	0.5 pH unit	Methanol : H_2O 1:1
		0.0 pH unit	H_2O
	advanced low-barrier	1.0 pH unit	H_2O
	advanced low-barrier	1.5 pH unit	H_2O

Figure 25. Synthetic Schemes for Representative Onco-Tools Containing Advanced pH-Switches

a)



b)

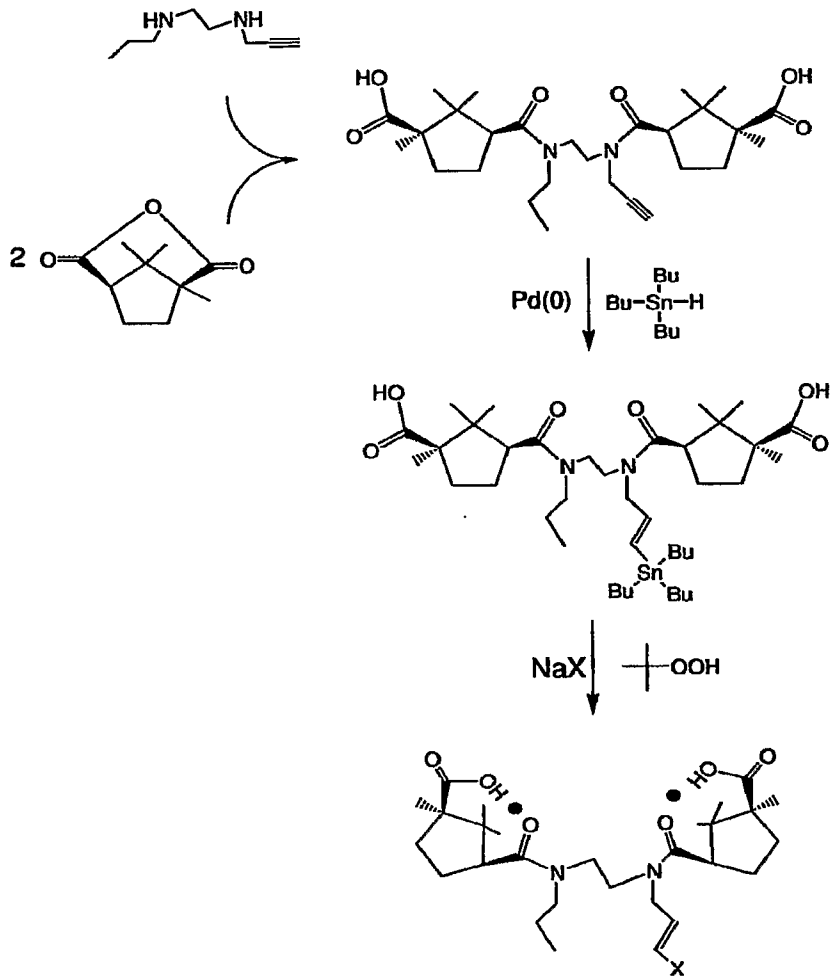
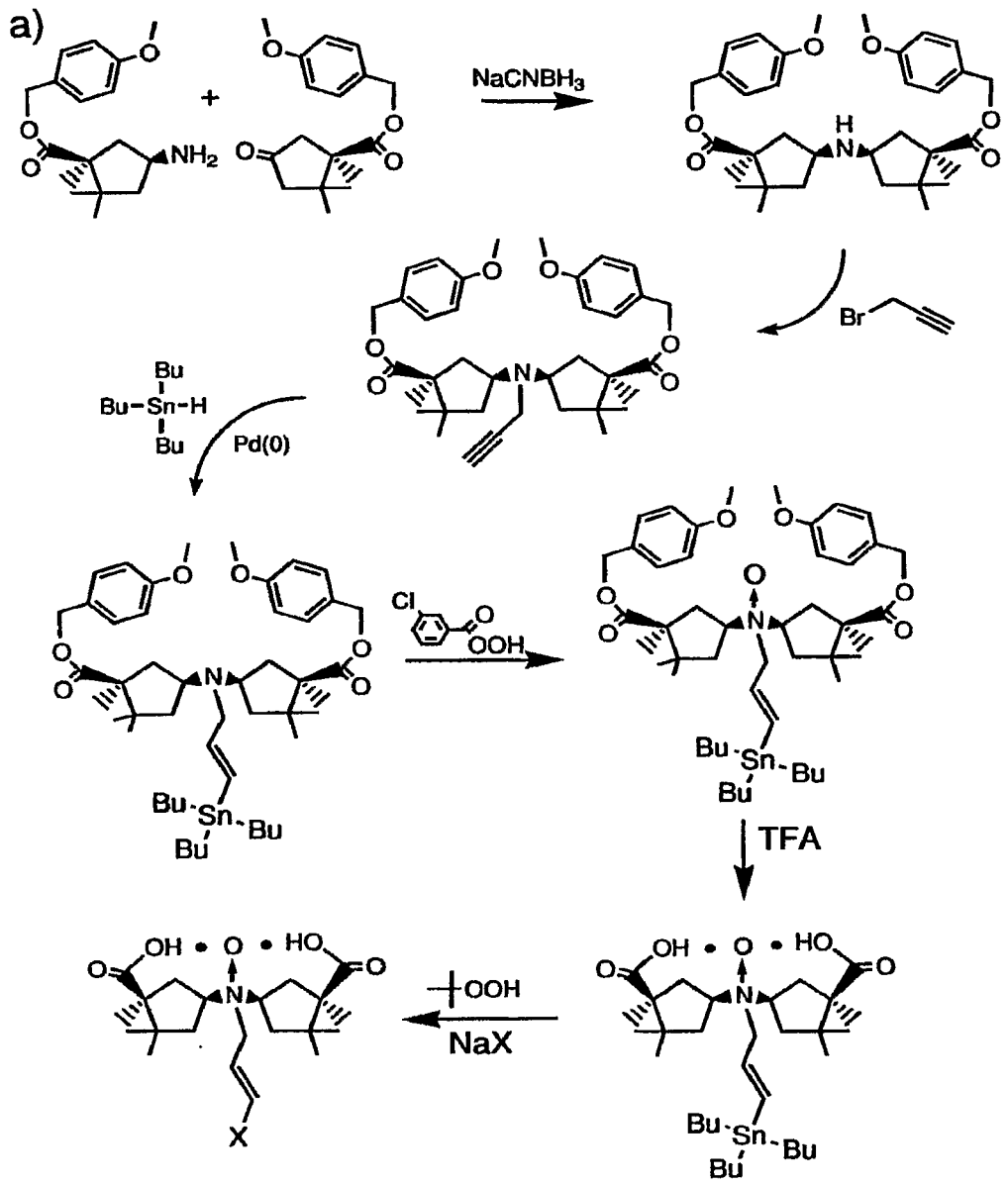
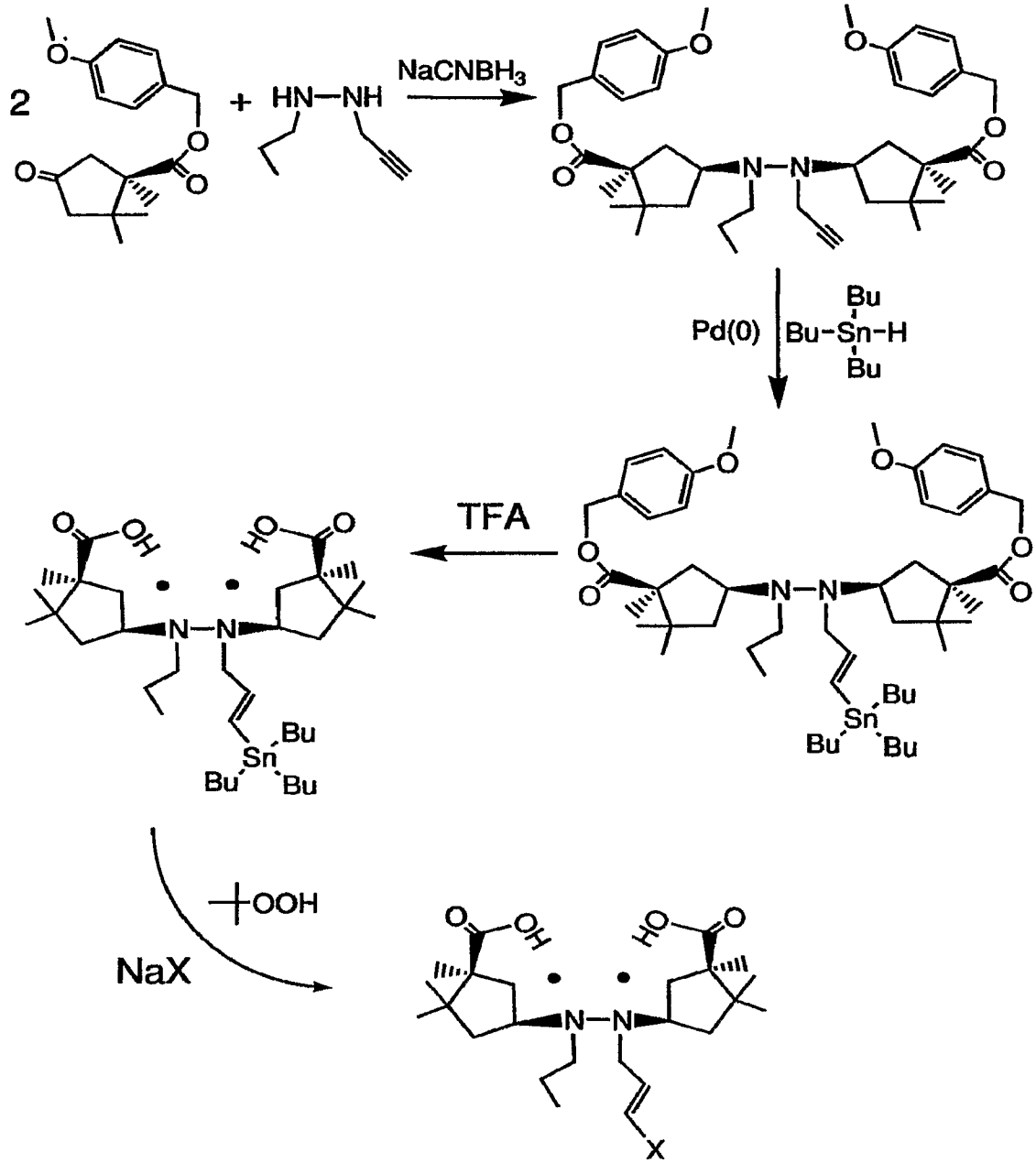
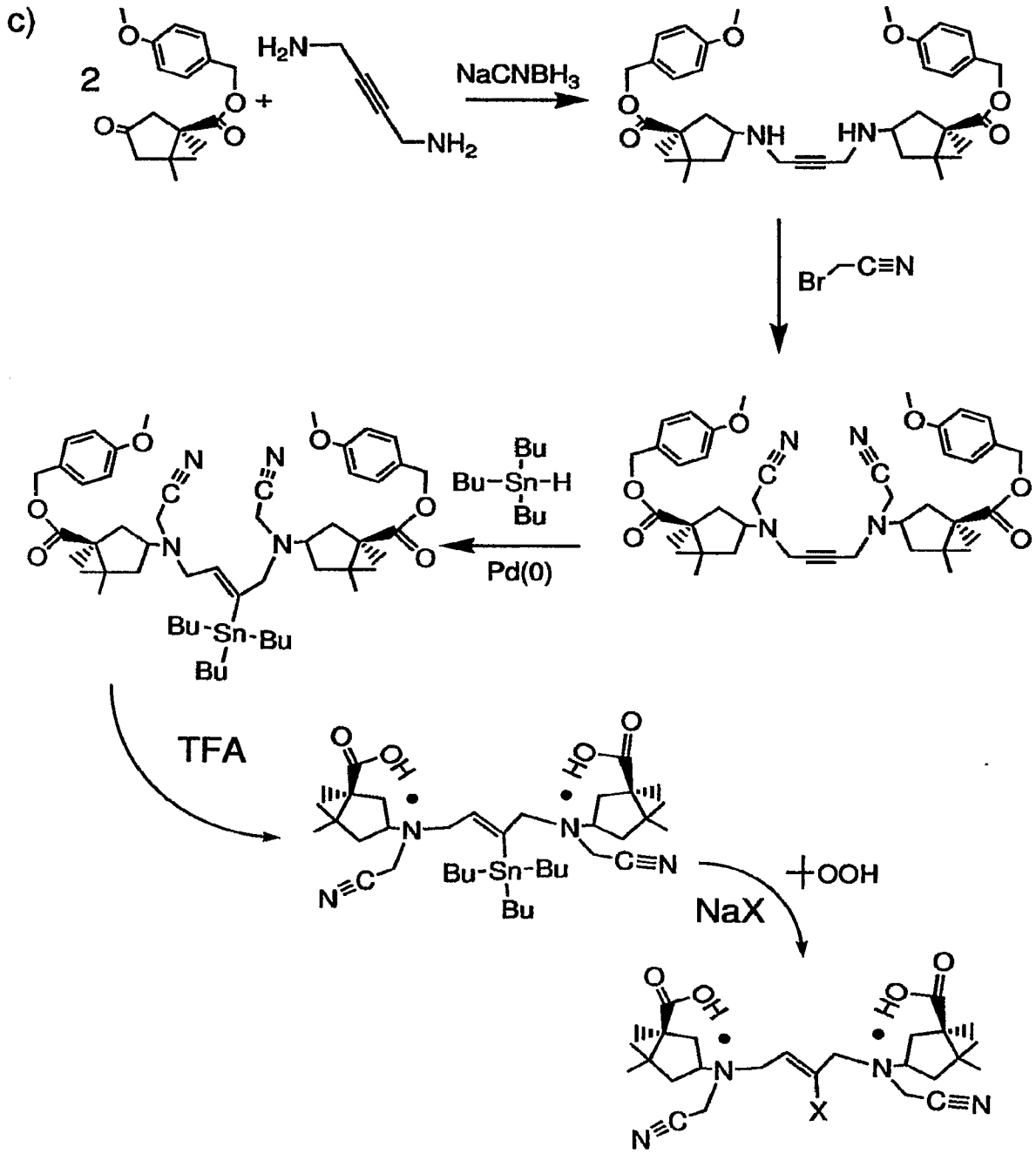


Figure 26. Synthetic Schemes for Representative Onco-Tools Containing Advanced pH-Switches Designed to Form a Low-Barrier H-Bond.



b)




c)
2
H_2N
NH_2
$NaCNBH_3$
NH
HN
Br
C≡N
Bu
Bu–Sn–H
Bu
Pd(0)
Bu-Sn-Bu
Bu
TFA
OH
HO
N
C≡N
Bu-Sn-Bu
Bu
C≡N
+OOH
NaX
X
C≡N

d)

