Photolabile linker for the synthesis of hydroxamic acids

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(54) **Title**: PHOTOLABILE LINKER FOR THE SYNTHESIS OF HYDROXAMIC ACIDS

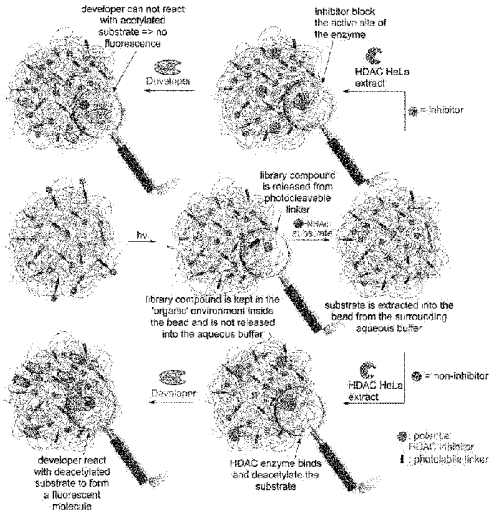

Figure 5

(57) **Abstract**: The present invention relates to a photolabile hydroxamate linker based on the o - nitroveratryl group and its application for multistep solid-phase synthesis and controlled photolytic release of hydroxamic acids. The invention provides a method for producing a solid support comprising a hydroxylamine - functionalized photolabile linker, and the so produced hydroxylamine - functionalized photolabile solid support. The invention further provides a method for synthesizing a one-bead-one compound library of hydroxamic acid derivatives on a photolabile linker, as well as a method for screening a library of hydroxamic acid derivatives.

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PHOTOLABILE LINKER FOR THE SYNTHESIS OF HYDROXAMIC ACIDS

BACKGROUND OF THE INVENTION

Field of the invention

The present invention relates to a photolabile hydroxamate linker based on the *o*-nitroveratryl group and its application for multistep solid-phase synthesis and controlled photolytic release of hydroxamic acids.

Prior art

Solid phase synthesis

Solid-phase organic synthesis originally developed by Merrifield for peptide synthesis, J. Am. Chem. Soc. 1963, 85, 2149–2154, has become an attractive synthetic technique that offers unique advantages over conventional solution phase chemistry, both in terms of purification and simplicity. Solid-phase synthesis may also be used for the preparation of non-peptide molecules. Both in academia and industry, there has been considerable interest in the solid-phase synthesis of combinatorial libraries for the identification of biologically active compounds in early drug discovery efforts (Moos et. al., Annu. Rep. Med. Chem. 1993, 28, 315-324).

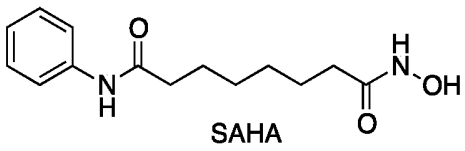
Hydroxamic acid derivatives

Hydroxamic acid derivatives represent an increasingly important class of biologically active compounds with a wide spectrum of antibacterial, antifungal, and anticancer properties. The hydroxamic acid moiety is present in numerous biologically active molecules of both natural and unnatural origin (Minucci et. al., Nature Reviews 2006, 6, 38-51). In addition, they are versatile intermediates that can be elaborated into interesting compounds for potential use in organic and medicinal chemistry (Iwasa et. al., Tetrahedron Lett. 2001, 42, 5897-5899; Van Maarseveen et. al., J. Med. Chem. 1992, 35, 3223-3230; Almeida et. al. Tetrahedron Lett. 1991, 32, 2671-2674; Hermkens et. al., J. Org. Chem. 1990, 55, 3998-4006; Almeida et al., Heterocycles 1989, 28, 653-656; Minucci, et al. Nature Reviews 2006, 6, 38-51, Whittaker, et al. Chem. Rev. 1999, 99, 2735-2776.

Hydroxamic acids are strong metal ion chelators and are known to inhibit enzymes having metal ions in their active sites. In particular, they have been identified as potent inhibitors of matrix metalloproteinases (MMPs), a family of zinc-dependent endoproteinases involved in both normal and diseased tissue remodeling, and as effective histone deacetylases (HDACs) inhibitors.

Overexpression of HDACs has been linked to gene silencing causing repression of anti-cancer genes.

SAHA is one example of a small molecule hydroxamic acid having anti-cancer activity. It was recently approved for treatment of cutaneous T cell lymphoma.



SAHA

Along these lines, there has been substantial interest in applying solid-phase combinatorial chemistry for the high-throughput generation of structurally diverse hydroxamic acids. Although hydroxamic acids may be obtained by direct cleavage of resin-bound esters with hydroxylamine (Dankwardt, Synlett 1998, 761; Thouin and Lubell, Tetrahedron Lett. 2000, 41, 457–460; Ho et al., J. Org. Chem. 2005, 70, 4873-4875; Dankwardt et al., Bioorg. Med. Chem. Lett. 2000, 10, 2513-2516), this approach does not give reproducible results (Floyd et al., Tetrahedron Letters 1996, 8045-8048; Dankwardt, Synlett 1998, 7, 761). Furthermore, this strategy often requires an excess of hydroxylamine and/or base additive, and due to poor solubility of $NH_2OH.HCl$ in most organic solvents, high-boiling DMF is the preferred solvent. Altogether these factors complicate post cleavage work-up and product purification. Alternatively, the use of protected hydroxylamine derivatives has been suggested, (Chen and Spatola, Tetrahedron Lett. 1997, 38, 1511-1514; Golebiowski and Klopfenstein, Tetrahedron Lett. 1998, 39, 3397–3400; Zhang et al., J. Comb. Chem. 2001, 3, 151-153; Sasubilli and Gutheil, J. Comb. Chem. 2004, 6, 911-915), but this strategy suffers from post-cleavage deprotection and purification steps.

A number of approaches involving resin-bound hydroxylamine linkers have been reported. These generally involve immobilization of the hydroxylamine group

through an *O*-linkage (Floyd et al., Tetrahedron Letters 1996, 37, 8045-8048; Richter and Desai, Tetrahedron Letters 1997, 38, 321-322; Mellor et al., Tetrahedron Letters 1997, 38, 3311-3314; Bauer et al., Tetrahedron Letters 1997, 38, 7233-7236; Khan and Grinstaff, Tetrahedron Letters 1998, 39, 8031-8034; Grigg et al., Tetrahedron Lett. 1999, 40, 7709-7711; Ede et al., Lett. Pept. Sci. 1999, 6, 157-163; Barlaam et al., J. Tetrahedron 1999, 55, 7221-7232; Mellor and Chan, Chem. Commun. 1997, 20, 2005-2006), but also *N*-linked strategies have been published (Ngu and Patel, J. Org. Chem. 1997, 62, 7088-7089; Gazal et al., J. Peptide Res. 2005, 66, 324–332). A synthetic disadvantage of these linkers may be the use of acidic reaction conditions for liberation of material from the solid support, since hydroxamic acids are sensitive to acidic degradation. Furthermore, some protecting groups, e.g., Boc and Trt, are generally not compatible with acids (Barany and Merrifield, J. Am. Chem. Soc. 1977, 99, 7363 –7365). The use of acid-labile linker strategies also limits the range of chemical transformations applicable during multistep synthesis of hydroxamic acid derivatives. Thus, other cleavage principles are required to provide new opportunities, such as synthetic methods relying on acidic reaction conditions, for the synthesis of chemically diverse hydroxamic acids.

US 2003/0013910 and US 6,281,245 disclose methods of synthesizing hydroxamic acid derivatives and Krchnak discusses chemical strategies developed for the solid-phase synthesis of hydroxamates (Mini-Reviews in Medicinal Chemistry, 2006, 6, 27-36).

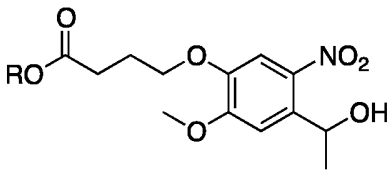
Photolabile linkers

Photolysis offers a method for cleavage which is fully orthogonal to chemical methods (James, Tetrahedron 1999, 55, 4855-4946). Furthermore, photolytic cleavage offers exceedingly mild conditions, which are attractive for direct applications in biological screening where contamination with cleavage reagents is undesired.

The 4,5-dialkoxy-2-nitrobenzyl group (Rich and Gurwara, J. Chem. Soc., Chem. Commun. 1973, 610-611) was pioneered in photolabile linkers by Yoo and Greenberg (J. Org. Chem. 1995, 60, 3358-3364) and Holmes and Jones (J. Org. Chem. 1995, 60, 2318-2319), for the solid-phase synthesis of oligonucleotides and

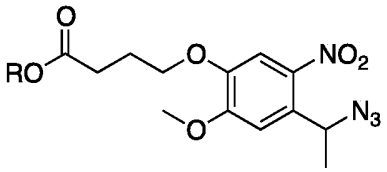
peptides, respectively. Photolabile linkers have been made to release a variety of functionalized molecules from solid supports, such as carboxamides, sulphonamides, carboxylic acids, alcohols, and amines. For an overview on photolabile linkers for solid-phase organic synthesis, see: Scott: Linker Strategies in Solid-Phase Organic Synthesis 2009, John Wiley and Sons.

Whitehouse et al, Tetrahedron Letters, 1997, 38(45) 7851-7852 discloses a photolabile linker for solid-phase organic synthesis having the structure:



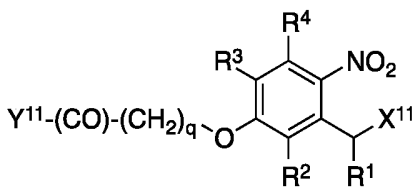
where R is hydrogen for use in solid-phase synthesis of carboxylic acids.

Qvortrup and Nielsen, Chem. Commun. 2011, 47, 3278-3280 disclose an azido-functionalized photolabile linker having the following structure:

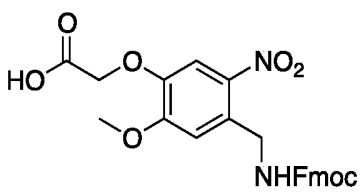


where R is hydrogen or ethyl for use in solid-phase synthesis of 4-substituted NH-1,2,3-triazoles.

WO 96/00378 discloses photolabile linkers for use in solid-phase synthesis, for example in the synthesis of small molecule and peptide libraries. The photolabile linking group is represented by the formula:

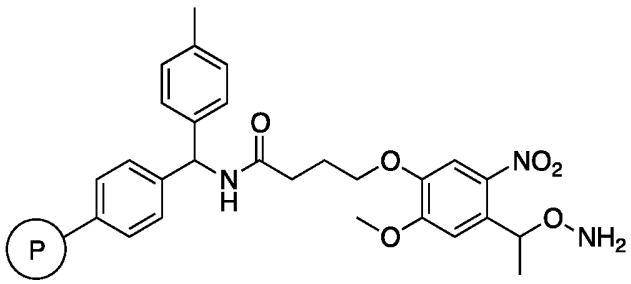


for example:



for use in solid-phase synthesis of carboxamides.

WO 96/262323 discloses linkers carrying a hydroxylamine or protected hydroxylamine group for use in solid-phase synthesis, where the linkers are chemically or photolytically cleavable. Example 5 discloses a photolabile linker on a solid support:



However, WO 96/262323 does not disclose a photolabile linker according to the present invention for the synthesis and release of a class of hydroxamic acids, which linker provides a simple and very efficient tool for attachment to any suitable solid support without the need for solid-phase hydroxylamination reaction steps to functionalize the linker.

SUMMARY OF THE INVENTION

The present invention relates to a photolabile linker based on the *o*-nitroveratryl group which linker is capable of releasing hydroxamic acids upon UV irradiation from a solid support. Furthermore the linker unit can be applied in a multi-detachable fashion. By simply varying the solvent, photolysis can be controlled to mediate either C-O or C-N bond cleavage, and thereby allow the controlled release of either hydroxamic acid or carboxamide derivatives, respectively. This strategy may introduce further diversity into target molecules and compound libraries. The linker further provides a possibility of screening released hydroxamic acid derivatives in situ, i.e. testing the released derivatives when still present in a solid support, such as beads.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Experimental setup used in photolysis reactions.

Figure 2: UV spectra for photolabile construct 15 in different solvents (0.05 mM)

Figure 3: Product formation resulting from photolysis of 15 at 360 nm under variation of solvent.

Figure 4: Illustration of the "split-and-mix" synthesis.

Figure 5: Illustration of the "in-bead" screening technology HDAC used in a screen of putatively active HDAC inhibitors.

Figure 6: Illustration of a post-screening structure determination strategy.

Figure 7: SAHA-containing beads (25c) showing HDAC-inhibitory activity when subjected to the "in-bead" HDAC-inhibition assay. When the photolysis step was omitted no SAHA was released and no quenching of HDAC-activity was observed. Beads functionalized with a ligand devoid of HDAC-inhibitory activity (25d) resulted in no quenching of fluorescence following photolysis.

Figure 8: Representative microscopy images of small, bead-based library (25a-h) subjected to in-bead HDAC-inhibition assay with a photolysis time of 2 min.

Figure 9: Representative microscopy images of small, bead-based library (25a-h) subjected to in-bead HDAC-inhibition assay with a photolysis time of 0.5 min.

Figure 10: Microscopy pictures of SAHA-containing beads (25c) resulting from in-bead HDAC-inhibition assay with a photolysis times of 5 s, 1 min and 5 min, respectively.

DETAILED DESCRIPTION OF THE INVENTION

List of Definitions

Protective group refers to a chemical group that exhibits the following characteristics: (1) reacts selectively with the desired functionality in good yield to give a derivative that is stable to the projected reactions for which protection is desired; (2) can be selectively removed from the derivatized solid support to yield the desired functionality; and (3) is removable in good yields by reagents compatible with the other functional group(s) generated in such projected reactions. Examples of protecting groups can be found in Greene et al. (1991)

Protective Groups in Organic Synthesis, 2nd Ed. (John Wiley & Sons, Inc., New York). Preferred examples are Fmoc, Boc, Trt, Alloc, *t*Bu, Cbz, Bn, SiR_3.

Fmoc = fluorenylmethyloxycarbonyl – removed by base, such as piperidine.

Boc = t-butyloxycarbonyl – removed by acid, such as HCl and CF_3COOH.

Trt = trityl – removed by acid, such as HCl and CF_3COOH

Alloc = allyloxycarbonyl – removed by tetrakis(triphenylphosphine)palladium(0) in a mixture of methylene chloride, acetic acid, and *N*-Methylmorpholine

*t*Bu = *tert*-butyl - removed by acid, such as HCl and CF_3COOH.

Cbz = carbobenzyloxy - removed by hydrogenolysis.

Bn = benzyl - removed by hydrogenolysis.

SiR_3, where R can be combinations of different groups. Common silyl protective groups are trimethylsilyl (TMS), *tert*-butyldiphenylsilyl (TBDPS), *tert*-butyldimethylsilyl (TBS/TBDMS) and triisopropylsilyl (TIPS), [2-(trimethylsilyl)ethoxy]methyl (SEM) – removed with acids or fluorides such as HF and tetra-*n*-butylammonium fluoride. Larger R-substituents increase resistance to hydrolysis.

Rink-linker = 2-(4-(amino(2,4-dimethoxyphenyl)methyl)phenoxy)acetic acid

NEM: *N*-ethyl morpholine.

PEGA: polyethylene glycol dimethyl acrylamide.

TBTU: *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluorobrate

HATU: *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate

TLC: Thin layer chromatography

HFIP: hexaflouroisopropanol

TFA: trifluoroacetic acid

HPLC: High-performance liquid chromatography

HDAC: histone deacetylase

pyBOP: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

DIPEA: *N*,*N*, Diisopropylethylamine

DMF: Dimethylformamide

Dox: Doxorubicin

SPPS: solid-phase peptide synthesis

Physicochemical or biological response: Any property that is measurable whose value describes a chemical or biological systems state. The changes in the physicochemical or biological responses of a system can be used to describe its transformations. The measurable values may include, but are not limited to: fluorescence (turn on/off fluorescence), chemiluminescence, absorbance, concentration, electric properties, pH, and the like.

Chemical or biological system: an integrated structure of components and subsystems capable of performing, in aggregate, one or more specific functions. A chemical or biological system may include, but are not limited to: binding of a ligand for a receptor of interest (e.g. GPCR), inhibition of an enzyme (e.g. HDAC), disruption of a protein/protein interaction (e.g. DNA replication), catalysis of a chemical transformation, and the like.

Activating group: refers to a group which, when attached to a particular functional group, renders that site more reactive toward covalent bond formation with a second functional group. The group of activating groups which are useful for a carboxylic acid include simple ester groups, anhydrides, and acid chlorides. The ester groups include alkyl, aryl and alkenyl esters and in particular esters of 4-nitrophenol, N-hydroxysuccinimide, N-hydroxybenzotriazole, and pentafluorophenol. Other activating groups are known to those skilled in the art.

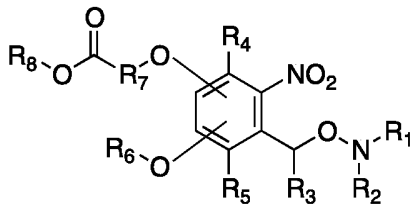
Chemical group: a chemical entity for example a building block in the synthesis which, prior to attachment, has one reactive functional group appropriate for attachment to a chemical group on the solid support, and one or more optionally

protected functional group appropriate for later further functionalization, e.g. amine-protected amino acids with activated carboxylic acid groups.

Combinatorial chemistry: Ordered strategy for the synthesis of diverse compounds by sequential addition of reagents, which leads to the generation of large chemical libraries. Thus, combinatorial chemistry refers to the systematic and repetitive, covalent connection of a set of different 'chemical entities' of varying structures to each other to yield large arrays of diverse molecular entities.

Chemical library: An intentionally created collection of differing molecules which can be prepared either synthetically or biosynthetically and screened in a chemical or biological system for a physicochemical or biological response, e.g. a biological activity.

In a first aspect, the present invention concerns a compound with the general formula I:



wherein R_1 and R_2 are the same or different and represent hydrogen, C_1-C_8 alkyl, aryl, heteroaryl, C_1-C_8 carboxyalkyl, carboxyaryl or arylalkyl,

R_3 is hydrogen, C_1-C_8 alkyl, phenyl or mono- or multiply-substituted phenyl, wherein the substitutions are the same or different and represent hydrogen, C_1-C_8 alkyl, C_1-C_8 alkoxy, halogen, nitrile or nitro,

R_4 and R_5 are the same or different and represent hydrogen, C_1-C_8 alkyl, C_1-C_8 alkoxy, aryl, heteroaryl, halogen, nitrile or nitro,

R_6 and R_7 are the same or different and represent C_1-C_8 alkyl, aryl, heteroaryl or -$(CH_2$-CH_2-$O)$-$_n$, where n is a integer from 1 to 100, and

R_8 is hydrogen, C_1-C_8 alkyl or aryl,

which compound finds use as a unique photolabile linker for the solid phase synthesis of hydroxamic acid derivatives.

In one embodiment R_1 is an amino protecting group, such as Fmoc or Boc and R_2 is hydrogen.

"C_1-C_8 alkyl" means a cyclic, branched, or straight chain chemical group containing 1-8 carbon atoms and containing only carbon and hydrogen. Examples are methyl, ethyl, propyl, iso-propyl, butyl and tert-butyl.

"C_1-C_8 alkoxy" refers to the group alkyl-O-, preferred examples are methoxy and ethoxy.

"aryl" means an aromatic carbocyclic group having a single ring (e.g. phenyl) or multiple condensed rings (e.g. naphthyl or anthracenyl), which can optionally be unsubstituted or substituted with amino, hydroxyl, C_1-C_8 alkyl, C_1-C_8 alkoxy, aryloxy, halo, mercapto, and other substituents. Preferred examples include phenyl, 1-naphthyl and 2-naphthyl.

"heteroaryl" means a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g. pyridyl or furyl) or multiple condensed rings (e.g. indolizinyl or benzothienyl) and having at least one hetero atom, such as N, O or S, within the ring, which can optionally be unsubstituted or substituted with amino, hydroxyl, C_1-C_8 alkyl, alkoxy, halo, mercapto and other substituents, preferred examples are 2-pyridyl and 2-quinolinyl.

"aryloxy" means the group aryl-O-, preferred examples are phenoxy and 2-naphthalenyloxy.

"heteroaryloxy" means the group heteroaryl-O-, preferred examples are 2-pyridinyloxy and 2-quinolinyloxy.

"Carboxy" or "carboxyl" means the –R'(COOH) where R' is C_1-C_8 alkyl, aryl, arylalkyl, heteroaryl.

"C_1-C_8 carboxyalkyl" means the group –(CO)-R' where R' is alkyl containing 1-8 carbon atoms.

"carboxyaryl" means the group –(CO)-R' where R' is aryl, heteroaryl, substituted aryl or substituted heteroaryl.

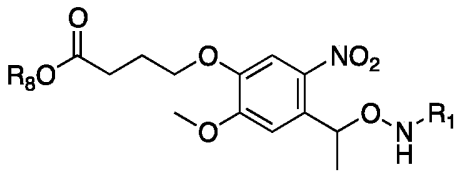
"arylalkyl" means the groups R'-aryl and R'-heteroaryl where R' is a cyclic, straight-chain or branched alkyl chain, examples are benzyl and furfuryl.

"halogen" means fluorine, chlorine, bromine or iodine.

$-(CH_2-CH_2-O)-_n$, refers to an oligomer or polymer of ethylene oxide, where n is an integer from 1 to 100, preferably n=1-50 or n=1-10.

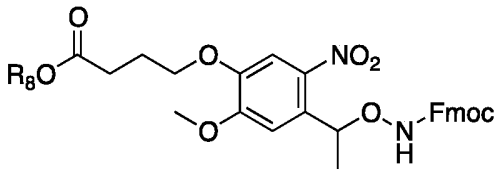
The compounds according to the present invention may be prepared in many different ways following standard procedures in organic synthesis. The person skilled in the art would readily know how to synthesize compounds according to formula I.

In one embodiment, preferred compounds of the present invention have the formula II:



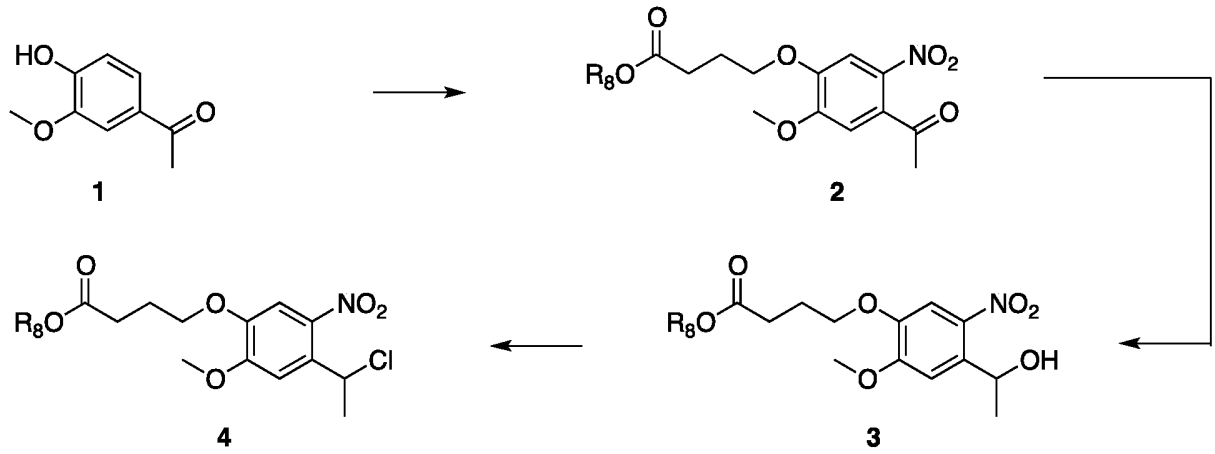
wherein R_1 is a protecting group, such as Boc, Fmoc, Alloc, Cbz, Bn and R_8 is hydrogen or C_1-C_8 alkyl.

In another embodiment, preferred compounds of the present invention have the formula III:

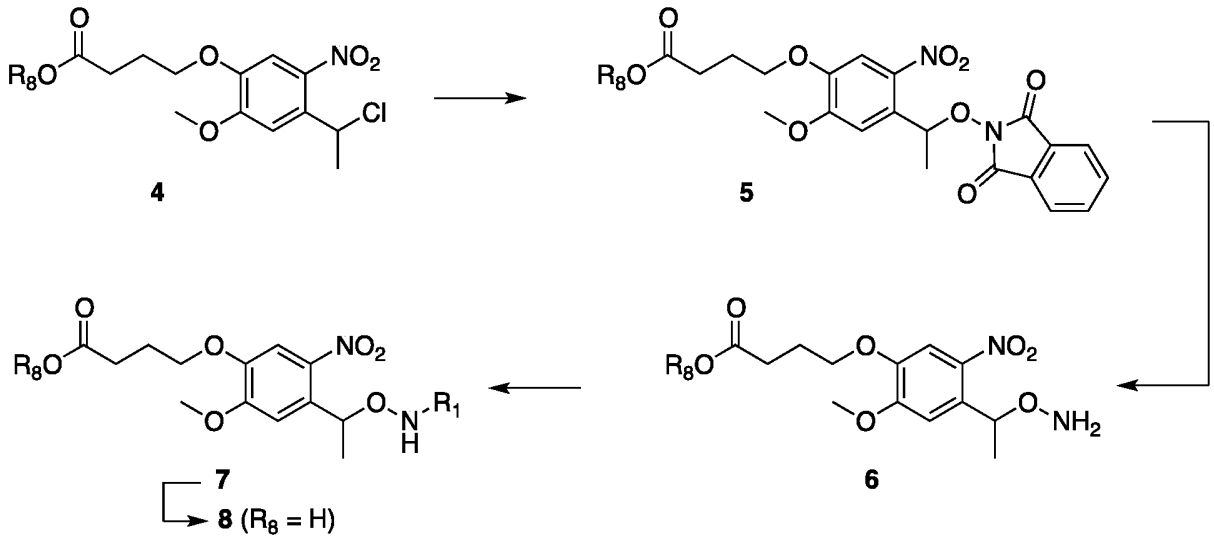


wherein R_8 is hydrogen, methyl or ethyl.

By way of example, one class of the compounds according to the present invention may be prepared starting from acetovanilone 1 from which ketone 2 can be prepared in a few high-yielding steps. Reduction of the ketone to the corresponding alcohol, followed by chlorination with thionylchloride in CH_2Cl_2 affords the key intermediate chloride 4.



Substitution of chloride can be effected by reaction with *N*-hydroxyphthalimide to give 5. Treatment of 5 with hydrazine removes the phthalimido group to give 6, which can then be protected with a protecting group R_1 (e.g. Fmoc) to give the protected hydroxylamine-ester 7. Selective hydrolysis of the ester group R_8 may be accomplished by any suitable chemical or biological hydrolysis process, for example by use of an appropriate esterase, thus affording the R_1 (e.g. Fmoc)-protected hydroxylamine-functionalized carboxylic acid linker 8. Novozyme 435 is one example of a suitable esterase for a selective removal of the ester group R_8.



In another aspect, the present invention concerns a method for producing a hydroxylamine-functionalized photolabile solid support comprising an attachment reaction and subsequent deprotection of the linker immobilized on a solid support.

The solid support

Nature of the solid support: Solid supports that can be functionalized with the linker may be of any shape or size, such as roughly spherical or a planer surface. The solid supports need not necessarily be homogenous in size, shape or composition; although the solid supports usually and preferably will be uniform.

Solid supports may consist of many materials, limited primarily by capacity for derivatization to attach any of a number of chemically reactive groups and compatibility with the synthetic chemistry used for linker attachment and/or synthesis. Suitable solid support materials typically will be the type of material commonly used in peptide and polymer synthesis. They include polymeric organic substrates, for example polystyrene, polypropylene, polyethylene glycol, polyacrylamide, cellulose and inorganic substrates, for example glass, silica gel or gold and other colloidal metal particles.

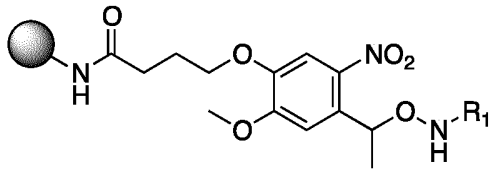
The chemically reactive groups with which such solid supports may be derivatized are those commonly used for solid phase synthesis, i.e., amino and hydroxyl.

For the "in-bead" synthesis and screening strategy, the solid support is preferably composed by polymeric beads, limited primarily by capacity for swelling, light permeability and the capacity for derivatization to attach any of a number of chemically reactive groups as well as compatibility with the synthetic chemistry used for linker attachment and/or synthesis. Suitable solid support materials typically will be the type of material commonly used in peptide and polymer synthesis. To improve swelling properties quite porous beads, resins, or other supports work well and are often preferable. Particularly preferred materials include polystyrene, polypropylene, polyethylene glycol and polyacrylamide resins, e.g. TentaGel® or Chemmatrix®.

Immobilization: The choice of functionality used for binding the linker to the solid support will depend on the type of solid support. Conditions for coupling monomers to solid supports through a wide variety of functional groups are known. For example, the carboxyl group of the linker can be activated by converting it to the corresponding –COP group wherein P is an activating group as defined above. This can then be coupled to an amino or hydroxyl group of the solid support.

The hydroxylamine-functionalized photolabile solid support

In one embodiment, the hydroxylamine-functionalized photolabile solid support has the following formula:

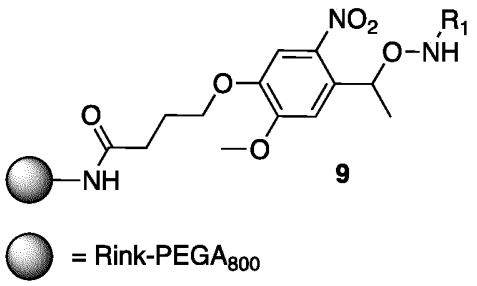


wherein R_1 is a protecting group, e.g. Alloc, Cbz, Bn, Boc or Fmoc and  represents a solid support, optionally including a spacer and/or functionalized with one or more secondary cleavable linkers. In the case of the solid support being functionalized with other cleavable linker(s), such additional linker(s) is/are either chemically or photolytically cleavable. Two or more differently cleavable linkers may be used to release portions of the synthesized compounds in subsequent cleaving steps to allow testing in one or more chemical or biological systems for one or more

physicochemical and/or biological responses followed by release of remaining compounds for identification of the compound(s) immobilized on positive solid support. Such linker(s) used in addition to the linker of the present invention are known in the art and chosen either from a commercial source or synthesized for this particular purpose.

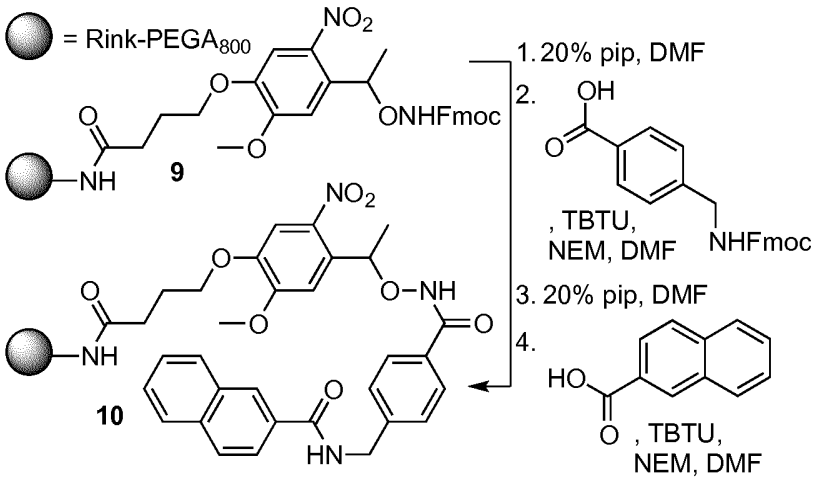
In one specific embodiment the hydroxylamine-functionalized photolabile solid support can be obtained by chemical coupling to immobilize the hydroxamic acid-releasing linker 8 to a suitable amino-functionalized solid support, such as a bead. Said solid support may be amino-functionalized by way of a Rink linker and/or *N*-terminal peptide sequences, including bromo-substituted amino acid residues.

For example, the Rink linker can be attached to the commercially available amino solid support ($PEGA_{800}$) in an *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluroniumtetra-fluorobrate(TBTU)-mediated coupling, followed by Fmoc-deprotection and coupling of linker 8, to afford the hydroxylamine-functionalized photolabile solid support 9.



9

The functionality of the present invention is illustrated in a small molecule model system, where the hydroxamate-functionalized photolabile solid support 10 is synthesized using standard TBTU-mediated peptide coupling reactions:



Photolytic cleavage

Photolytic cleavage is carried out on the hydroxamate-functionalized photolabile solid support suspended in appropriate solvents by irradiation for an amount of time to allow the desired cleavage to take place. By changing the time of illumination, from 0 to 100 % of the photolabile bonds are cleaved. When the present invention is used as a production platform, it may be desirable to release as much as possible in one step by choosing a sufficiently long time-length of illumination. A quality check of the synthesized compound may be incorporated by release of a small portion before release of the whole lot. In contrast, when the present invention is used in synthesis and screening of a library, it is desirable to control the amount of cleavage taking place in each of two or more consecutive cleavage steps. If more tests on the same synthesized hydroxamic acid derivatives are desired, sequential release provides a good opportunity for such testing or screening. The present invention provides a unique tool for such multiple testing/screening scenarios. For example, in a first step less than 90% is released, for example 1-90%, 5-50% or 5-30% of the hydroxamic acid derivatives are released from the solid support, e.g. inside beads for use in screening the library for physiochemical or biological responses by adding a (first) chemical or biological system to the solid support (the beads). Subsequent portions of the compounds in the library may be released in a second or further step for a second or further testing in a second or further chemical or biological system and finally for identification of the individual chemical structure of the hydroxamic acid

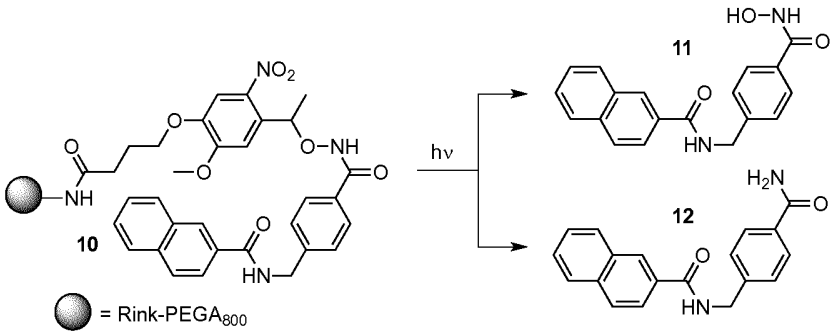
derivative(s) showing positive response(s). Each piece of solid support, e.g. bead may contain a sufficient amount of compounds for a post-screening hit identification. Photolytic release of compounds from active (positive) beads may for example also be used for a dose-response assay to validate the activity observed in a primary screening.

Alternatively, one or more additional linkers may be included in the hydroxylamine-functionalized photolabile solid support for additional chemical or photolytic cleavage. Such additional linkers comprise base-labile, acid-labile, metal-labile, safety-catch and photolabile linkers, known to persons skilled in the art. Other linkers may also be included in order to optimize and verify the attachment chemistry. Such linkers, such as for example the Rink linker, are known is the art.

To facilitate post-screening, such as MALDI-TOF MS sequencing of active beads, different spacers, such as a 4-bromophenylalanine spacer may be positioned in connection with the linker. Such spacers are known in the art.

The energy needed in the photolysis step to cleave the linker according to the present invention is provided by a 360 nm light source, for example a 400W LED UV-lamp. Figure 1 illustrates an experimental setup used in photolysis reactions.

For the release of synthesized compounds, e.g. from a library of functionalized small molecules, photolytic cleavage is carried out on a hydroxamate-functionalized photolabile solid support. In one example, set up to illustrate the present invention, the hydroxamate-functionalized photolabile solid support 9 is suspended in appropriate solvents and cleaved by irradiating for a certain time at room temperature with 360 nm light using a 400W LED UV-lamp. The resulting products, hydroxamic acid 11 and the carboxamide 12, result from C-O and N-O cleavage, respectively. When aqueous media is used, the hydroxamic acid is the predominant product.



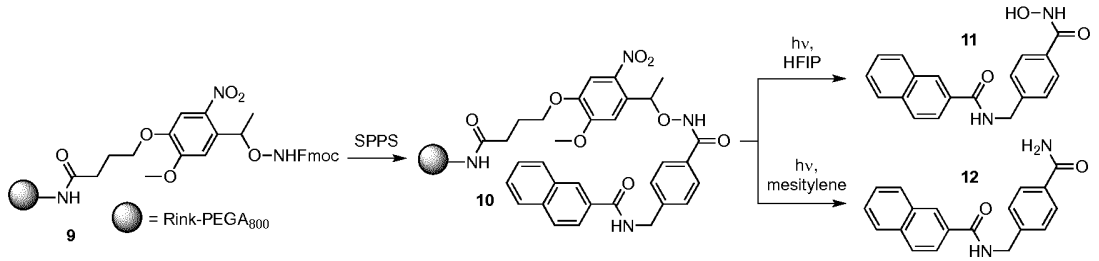
The solvent

The photolytic cleavage may result in a mixture of hydroxamic acid and carboxamide derivatives. By simply varying the solvent, photolysis can be controlled to mediate either C-O or N-O bond cleavage, and thereby allows the controlled release of either hydroxamic acid or carboxamide derivatives, respectively. This strategy may introduce further diversity into target molecules and compound libraries.

To investigate the solvent effects important for photolysis of the linker according to the present invention, the photolysis of immobilized hydroxamic acid derivative 10 was analyzed on the level of final product formation. The photoreaction was studied by photolyzing aliquots of the functionalized hydroxylamine-functionalized photolabile solid support 10 in various solvents and determining the product distribution via HPLC analysis.

Because the solvent also influences the swelling and solvation properties of the solid support, the results are only qualitative. While this technique did not allow a quantification of the amount of products formed, it did provide an expedient method to achieve the goal, namely the determination of the relative photoproduct formation. In Table 1, the product distribution profiles are listed. It is evident that the solvent has a strong influence on the carboxamide-hydroxamic acid ratio of the reaction and some general conclusions are possible. Polar solvents favor formation of the hydroxamic acid product 11, while apolar solvents mainly give the carboxamide product 12. In particular, the polar fluorinated alcohol, hexafluoroisopropanol (HFIP) with a high hydrogen-bond-donating ability leads to hydroxamic acid product 11 with high selectivity. This selectivity is superior to that

observed when Lewis acids are used as catalysts (see below). It is well known that HFIP is a weak Brønsted acid (pKa= 9.3), meaning that catalysis through proton transfer can be envisaged to favor the hydroxamic acid-generating pathway.



Apolar solvents favor formation of the carboxamide product 12. Inspection of the product pattern reveals that selectivity increases in the order mesitylene > xylene > toluene. Carrying out the reaction in mesitylene exclusively provides carboxamide product 12.

Entry	Solvent	Product **11**,**12**
a	pentane	28:72
b	cyclohexane	27:73
c	toluene	33:67
d	xylene	17:83
e	mesitylene	0:100
f	benzene	39:61
g	*i*PrOH	64:36
h	THF	44:56
i	MeOH/H_2O (1:4)	67:33
j	CH_3CN	70:30
k	DMF	60:40
l	H_2O	60:40
m	HFIP	98:2
n	hexafluorobenzene	22:78
o	1,3-bis(trifluromethyl)benzene	26:74
p	1,3-diethylbenzene	25:75
q	mesitylene/HFP (1:1)	98:2
r	mesitylene/HFP (3:1)	92:8
s	mesitylene/HFP (9:1)	85:15
t	0.1M TFA in mesitylene	65:35
u	0.1M TFA in H_2O	90:10
v	0.1M TFA in CH_3CN	89:11
x	0.005 M HCl in H_2O	100:0

TABLE 1: Distribution of products resulting from photolysis of 9 at 360 nm by varying the solvent and acidity of the solution. Photolytic cleavage was carried out for 0.5 h with a LED lamp (360 nm). Product distribution was determined by RP-HPLC (254 nm).

The effect of Lewis acid catalysis on the photoreaction of 10 has been investigated, see Table 2. The qualitative studies show that a wide range of Lewis acids favor the formation of the hydroxamic acid product. The most efficacious Lewis acid was BF_3, but also the activating effects of the salt $LiBF_4$ are noteworthy in that this represents quite mild conditions. Also small amounts of protic acid, such as HCl, TFA gave high selectivity towards formation of the hydroxamic acid product 11 (Table 1).

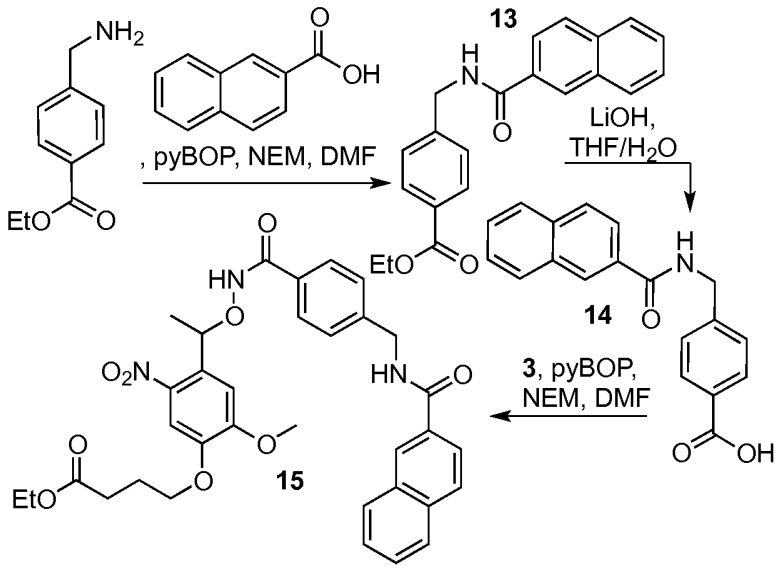
Entry	Solvent	Lewis acid	Product **11**,**12**
a	CH_3CN	0.1M LiBr	47:53
b	CH_3CN	0.1M $LiBF_4$	83:17
c	CH_3CN	0.1M $ZnCl_2$ in	57:43
d	CH_3CN	0.1 M $Sc(OTf)_3$	43:57
e[1]	THF	0.05 M $MgBr_2$	6:76(:18)[a]
f[2]	toluene	0.1 M $TiCl_4$	—
g[3]	heptane	0.1 M $SnCl_4$	—
h	CH_3CN	0.1 M BF_3,OEt_2	100:0
i	CH_3CN	0.05 M BF_3,OEt_2	86:14

TABLE 2: Relative product distribution for photolysis of 7 at 360 nm in the presence of Lewis acids. Photolytic cleavage was carried out for 0.5 h with a LED lamp (360 nm). Product distribution was determined by RP-HPLC (254 nm). [a]18% of the corresponding carboxylic acid was observed. [1]THF was used as solvent due to low solubility of $MgBr_2$ in CH_3CN. [2]$TiCl_4$ was used as a commercial stock solution in toluene. [3]$SnCl_4$ was used as a commercial stock solution in heptane.

To further investigate the photoreaction of the hydroxamate-functionalized compounds according to the present invention, the *o*-nitroveratryl derivative 15 was synthesized as a model compound for studying the photolysis in solution. In this way it was possible to identify the nature of by-products formed in the photolysis of a hydroxamate-functionalized *o*-nitroveratryl compound, illustrative of the compounds with general formula I, to gain a deeper mechanistic understanding. In this way, solution-phase photolysis experiments provide opportunities for studying the photolysis of hydroxamate-functionalized I compounds according to

the present invention without the potential influence of swelling and solvation properties of the solid support.

Synthesis of the model hydroxamate-functionalized *o*-nitroveratryl derivative 15 followed the scheme:



The UV spectra of the model derivative 15 were measured in a broad range of polar and apolar solvents and in solutions with either high or low acidity (Figure 2). In all cases, the typical absorption of the nitro-veratryl moiety with its characteristic maxima around 350 nm was observed. The differences in absorbance and extinction coefficients at the photolysis wavelength of 360 nm are only minor, which indicates that the absorbance of the nitroveratryl moiety is not substantially influenced by the nature of the solvent. This indicates that it is not the primary photo-excitation of the nitro group which determines the product ratios but more probably the kinetics and equilibrium position of the presumed intermediate aci-nitro compounds.

Product synthesis

In a further aspect, the present invention concerns a method, a production platform for synthesizing a hydroxamic acid derivative comprising:

a) coupling a compound/linker according to the present invention to a solid support,

b) coupling a chemical group to the immobilized compound/linker, and

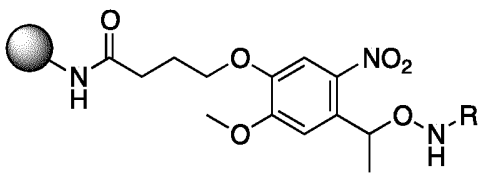
c) releasing said hydroxamic acid derivative from said solid support by photolytic cleavage.

The coupling of a chemical group may involve one or more steps in a synthesis of the desired hydroxamic acid derivative, i.e. one or more building blocks in the form of chemical groups may be coupled together during the synthesis on the photolabile linker of the present invention which again is coupled to the solid support in the first step.

Multistep solid-phase peptide synthesis may in this way be carried out on the present photolabile linker, and the resulting peptide hydroxamic acid be released by photolysis. Also multistep solid-phase oligonucleotide, oligosaccharide and other polymers may be synthesized by the present invention as derivatives and released by photolysis. In a preferred embodiment, the product platform may be used to produce desired hydroxamic acid derivatives of small molecules.

Synthesis of a specific hydroxamic acid compound may follow is this way and provides for an easy and reproducible synthesis of a desired product in high purity under neutral reaction conditions.

The present invention further concerns a hydroxylamine-functionalized compound immobilized on solid support through a photolabile linker having the formula:



wherein R is a chemical entity, i.e. the compound to be converted into a hydroxamine acid derivative and ● represents a solid support, optionally including a spacer and/or a second cleavable linker.

Hydroxamic acid derivative library

The hydroxylamine linker having the general formula I may serve as the starting point for the combinatorial synthesis of hydroxamic acid libraries.

As an example the hydroxylamine linker 8 was synthesized and employed for the parallel synthesis of a library of putative HDAC inhibitors (Table 3). A Rink linker was positioned between the solid support and the photolabile linker unit to optimize and verify attachment chemistry of linker 8 on the solid support.

Orthogonal and quantitative cleavage of the acid-labile Rink linker indicates the ratio of hydroxamate to unconverted photolabile hydroxylamine-linker, thus providing a measure of the loading efficiency.

After incubating the solid supports 16a-ae with TFA/CH_2Cl_2 (1:1) for 2 h, one major peak corresponding to cleavage of the Rink linker was generally observed, indicating high efficiency of the attachment chemistry of 8 and high stability of the photolabile unit 17 toward the TFA deprotection conditions normally used in standard peptide synthesis procedures.

Photolytic cleavage was carried out on an amount of solid support suspended in appropriate solvent by irradiating for 0.5-3 h at room temperature with 360 nm light using a 400W LED UV-lamp.

The possibility of selectively cleaving these compounds to give the hydroxamic acid and the carboxamide products, respectively, was demonstrated. Selected cleavage strategies are presented in Table 3. As seen in Table 3, the solid-phase strategy is very robust and applicable to a range of both aromatic and aliphatic hydroxamates. The released material was analyzed via UPLC/MS and comparison with known products indicated clean photolytic release of the desired hydroxamic acids and carboxamides, respectively. The liberated hydroxamic acids were recovered in high purity (47-95%) and satisfactory isolated yields (45-63%) (Table 3). To support the UPLC-MS evidence for product formation, larger scale synthesis and photo-release of two library compounds (11 and 18a) were carried out and crude photo-products were analyzed by 1H-NMR spectroscopy, showing clean photolytic release of the desired hydroxamic acids.

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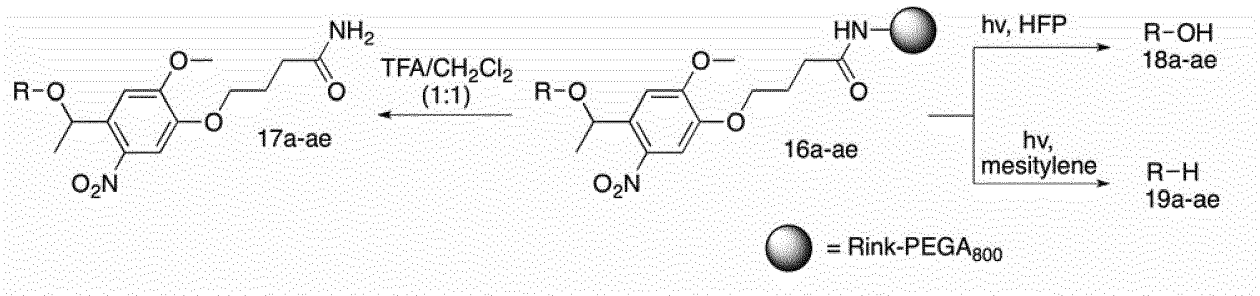
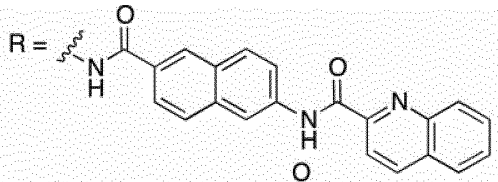
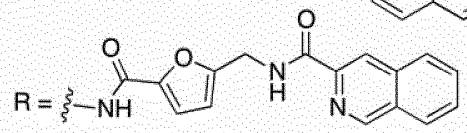
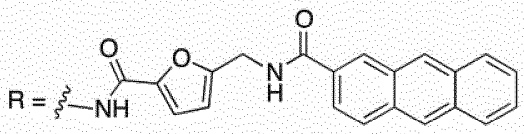
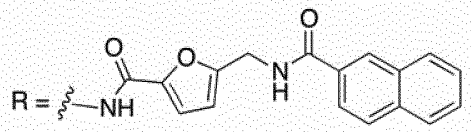
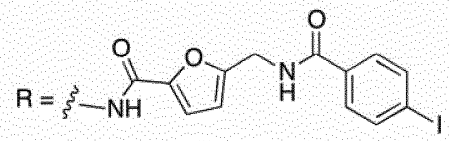
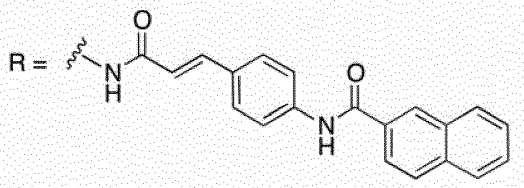
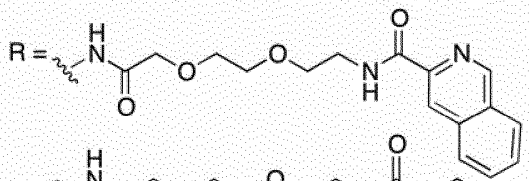
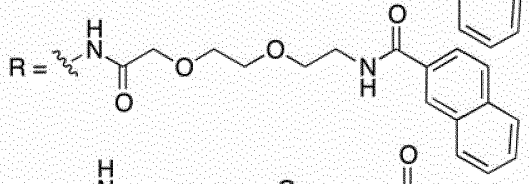
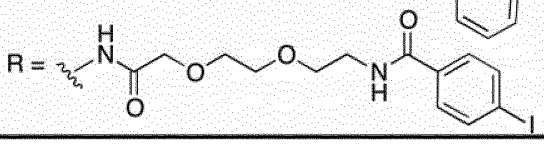
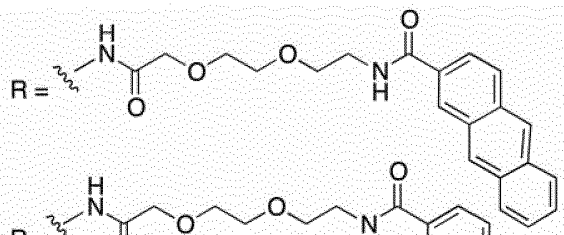
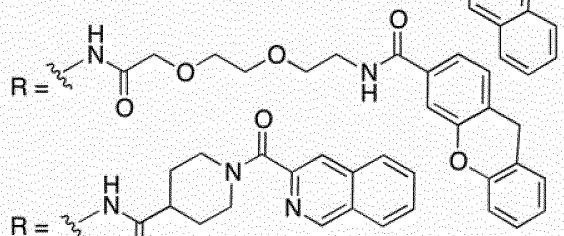
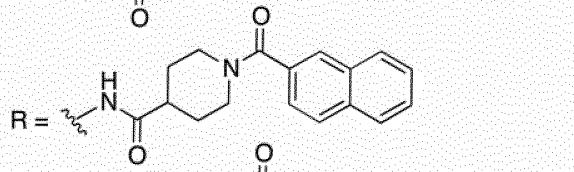
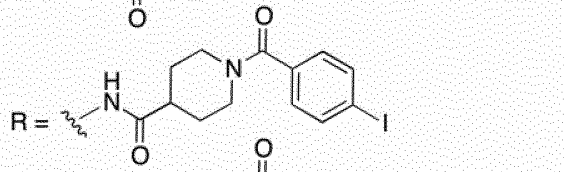
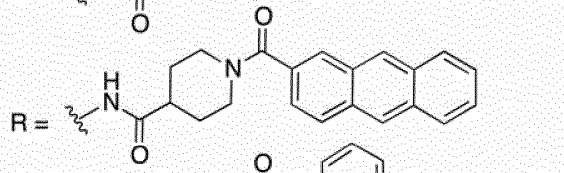
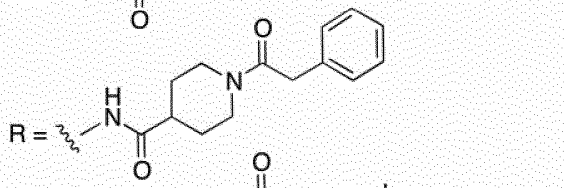
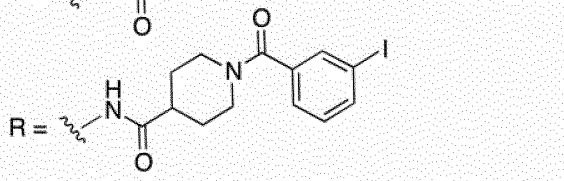
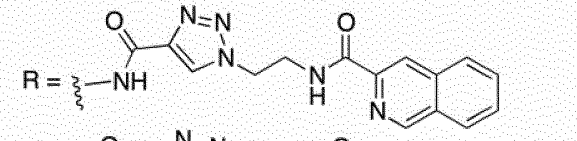
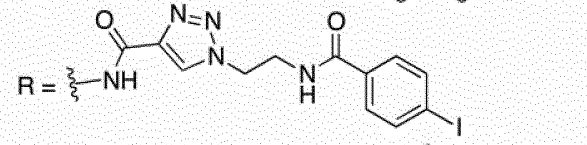
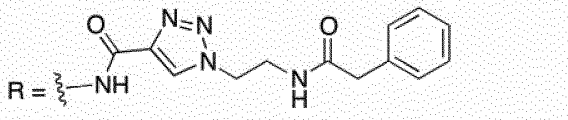


Entry	Substrate	Purity[a]	Yield[b]
a		17a: 85% 18a: >95% 19a: 83%	18a: 61%
b		17b: 42% 18b: 48% 19b:71%	18b: 54%
c		17c: 60% 18c: 51% 19c: 43%	18c: 60%
d		17d: >95% 18d: 91% 19d: 81%	18d: 54%
e		17e: 90% 18e: 85% 19e: 82%	18e: 48%
f		17f: 84% 18f: 91% 19f: 73%	18f: 62%
g		17g: 71% 18g: 92% 19g: 85%	18g: 58%
h		17h: 84% 18h: 94% 19h: 92%	18h: 63%
i		17i: 74% 18i: 87% 19i: 86%	18i: 45%

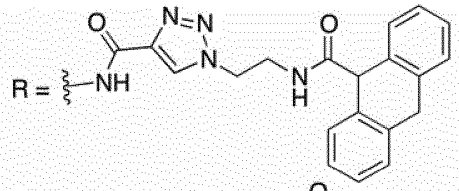
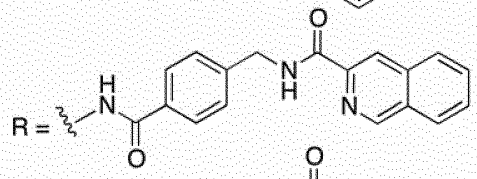
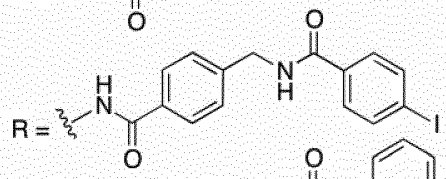
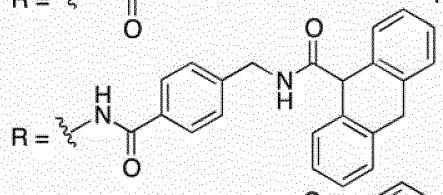
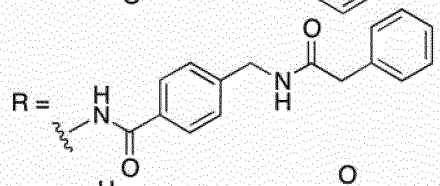
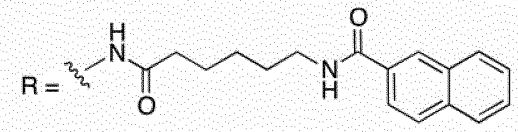
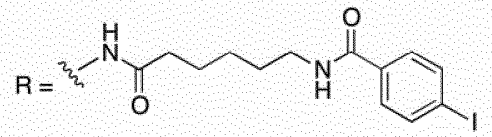
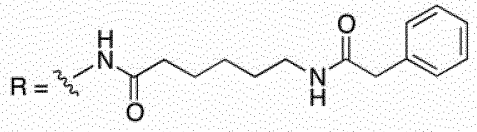
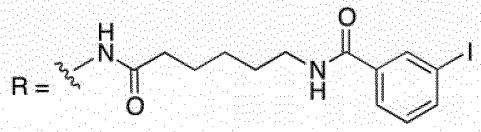
Photolytic cleavage was carried out for 2 h with an LED UV-lamp 400W (360 nm). Cleavage of the Rink linker was carried out with TFA/CH_2Cl_2 (1:1) for 2 h. [a] Purities were determined by RP-HPLC (254 nm). [b] Isolated yields after photolysis in HFIP.

Table 3 continued

Entry	Substrate	Purity[a]	Yield[b]
j		17j: 93% 18j: 95% 19j: 92%	18j: 47%
k		17k: 84% 18k: 47% 19k: 52%	18k: 53%
l		17l: 91% 18l: 93% 19l: 87%	18l: 62%
m		17m: 93% 18m: >95% 19m: 92%	18m: 51%
n		17n: 92% 18n: 85% 19n: 86%	18n: 53%
o		17o: 94% 18o: 92% 19o: 70%	18o: 48%
p		17p: 76% 18p: 85% 19p: 86%	18p: 50%
q		17q: 83% 18q: 76% 19q: 68%	18q: 54%
r		17r: 85% 18r: 93% 19r:82%	18r: 60%
s		17s: 74% 18s: 73% 19s: 68%	18s: 47%
u		17u: 82% 18u: 94% 19u: 92%	18u: 58%

Photolytic cleavage was carried out for 2 h with an LED UV-lamp 400W (360 nm). Cleavage of the Rink linker was carried out with TFA/CH_2Cl_2 (1:1) for 2 h. [a] Purities were determined by RP-HPLC (254 nm). [b] Isolated yields after photolysis in HFIP.

Table 3 continued

Entry	Substrate	Purity[a]	Yield[b]
t		17t: 74% 18t: >95% 19t: 82%	18t: 55%
v		17v: 76% 18v: >95% 19v: 91%	18v: 57%
x		17x: 85% 18x: >95% 19x: 92%	18x: 61%
y		17y: 69% 18y: 86% 19y: 78%	18y: 54%
z		17z: 72% 18z: 92% 19z: 91%	18z: 45%
aa		17aa: 92% 18aa: >95% 19aa: 75%	18aa: 47%
ab		17ab: 93% 18ab: >95% 19ab: 76%	18ab: 63%
ac		17ac: 91% 18ac: 71% 19ac: 81%	18ac: 57%
ad		17ad: 88% 18ad: >95% 19ad: 67%	18ad: 59%
ae		17ae: 89% 18ae: 76% 19ae: 80%	18ae: 62%

Photolytic cleavage was carried out for 2 h with an LED UV-lamp 400W (360 nm). Cleavage of the Rink linker was carried out with TFA/CH_2Cl_2 (1:1) for 2 h. [a] Purities were determined by RP-HPLC (254 nm). [b] Isolated yields after photolysis in HFIP.

TABLE 3: Library compounds synthesized on solid phase employing linker 8. Photolytic cleavage was carried out for 2 h with an LED UV-lamp 400W (360 nm). Cleavage of the Rink linker was carried out with TFA/CH_2Cl_2 (1:1) for 2h. [a] Purities were determined by RP-HPLC (254 nm). [b] Isolated yields after photolysis in HFIP.

Accordingly, the present invention discloses a novel method for synthesizing a library of hydroxamic acid derivatives, comprising:

a) coupling a compound/linker according to the present invention to a solid support,

b) removing the protecting group R_1,

c) coupling one or more different chemical entities to the immobilized compound/linker,

d) providing two or more different chemical groups and coupling them to the one or more chemical groups coupled to the solid support in step c), and

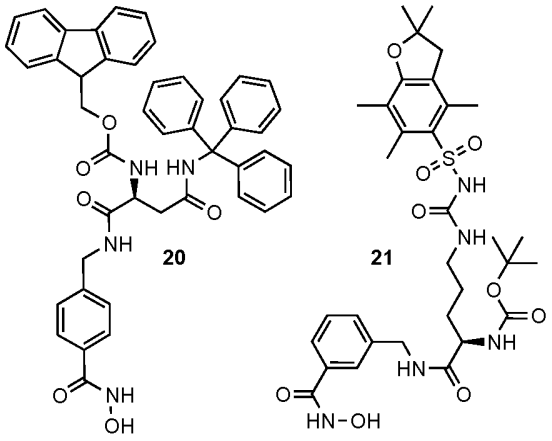
e) repeating the coupling step as many times as desired/necessary to obtain the desired library.

In one embodiment of the present invention, the steps of coupling different groups on the solid support are performed in a way to obtain coupling of the different chemical groups in a combinatorial fashion. The synthesis of one-bead-one-compound (OBOC) combinatorial libraries is useful for the discovery of bioactive compounds. The method is particularly attractive since hundreds of thousands of chemical compounds can be generated via split-pool synthesis within a short time. In addition, each compound is localized on an identifiable solid support such as an individual bead and therefore spatially addressable during hit identification. For example, to provide a mixture of a high number of different compounds in the library, the so-called "split-and-mix" synthesis may conveniently be used.

The "split-and-mix" synthesis is illustrated in Figure 4. A large assembly of beads is suspended in a suitable solvent in a parent container. The beads are provided with a photocleavable linker having a reactive site. The reactive site is protected by an optional protecting group. In a first step of the synthesis, the beads are divided for coupling into separate containers. The protecting groups are then removed and a first portion of the molecule to be synthesized is added to the various containers.

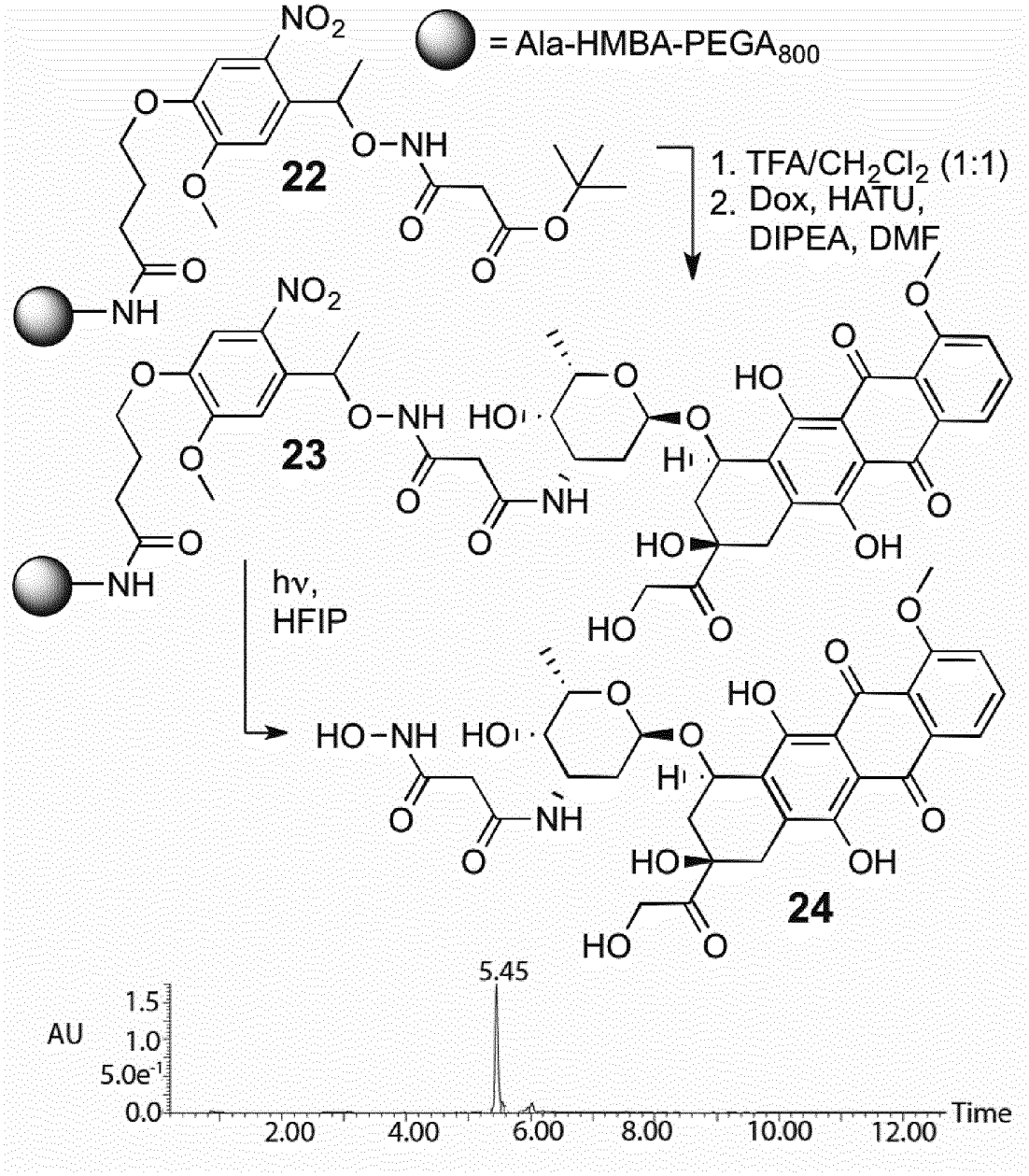
For the purpose of this brief description, the number of containers will be limited to three and the chemical entities denoted as A, B, C, D, E, and F. The protecting groups are then removed and a first portion of the molecule to be synthesized, i.e., the first chemical group, is added to each of the three containers (i.e., A is added to container 1, B is added to container 2 and C is added to container 3). Thereafter, the various beads are washed of excess reagents as appropriate, and remixed in a parent container. Again, it will be recognized that by virtue of the large number of beads utilized at the outset, there will similarly be a large number of beads randomly dispersed in the parent container, each functionalized with a particular first chemical group. Thereafter, the various beads are again divided for coupling in another group of three containers. The beads in the first container are deprotected and exposed to a second chemical group (D), while the beads in the second and third containers are coupled to chemical groups E and F respectively. Accordingly, molecules AD, BD, and CD will be present in the first container, while AE, BE, and CE will be present in the second container, and molecules AF, BF, and CF will be present in the third container. Each bead, however, will only display a single compound structure. Thus, all of the possible compounds formed from the first portions A, B, C, and the second portions, D, E, F are formed. The beads are then recombined into one container and additional steps such as are conducted to complete the synthesis of the combinatorial library.

While the linker 8 has been demonstrated to be stable towards both acidic and basic conditions, the utility of the linker for the synthesis of acid labile substrates has also been tested. Both hydroxamic acid-functionalized amino acid derivatives containing Fmoc- (20) and Boc- (21) protected α-amino groups and Trt-protected amide (20) and Pbf-protected guanidinium (21) side groups were successfully synthesized on the linker immobilized on a solid support and released, demonstrating the extraordinary chemical orthogonality of the linker in a hydroxylamine-functionalized photolabile solid support.



The potential of the linker according to the present invention, for the generation of more elaborate structures, is further demonstrated by the use of linker 8 to generate a hydroxamic acid-functionalized Doxorubicin (Dox) derivative. Dox is a highly potent anticancer agent. However, its application is limited by significant cardiotoxic side effects. Considerable work has therefore been undertaken to chemically modify Dox with the goal of reducing its systemic toxicity. Synthetic efforts in this context are hampered by the sensitivity of Dox to acidic and basic reaction conditions. Therefore, the efficient synthesis and release of a modified Dox derivative on the linker shows the potential of the linker for the generation of more elaborate structures in a combinatorial library format.

The use of linker 8 for the generation of a hydroxamic acid-functionalized Dox derivative was demonstrated by treating a hydroxylamine-functionalized photolabile solid support in a standard solid-phase peptide synthesis (SPPS) with mono-*tert*-butyl malonate. The *tert*-butyl protecting group was removed with TFA/CH_2Cl_2 (1:1) and the carboxylic acid functionalized photolabile solid support was treated with Dox in a O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyl-uroniumhexafluorophosphate (HATU)-mediated coupling to give 23. Rewardingly, the steps of synthesis and photolytic release of the complex hydroxamic acid-functionalized Dox-derivative 24 were very clean.



In bead screening

The screening of one-bead-one-compound (OBOC) combinatorial libraries is useful for the discovery of molecular interactions, such as inhibitors of enzymes and other molecular interactions, such as protein-protein interactions. The method is particularly attractive since thousands of chemical compounds can be generated via e.g. split-pool synthesis in short time. This approach requires that each compound localized on an individual bead is spatially addressable during hit identification. The basic idea of screening ligands directly on bead was recognized by Lam et al. as

early as 1991 (K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M.Kazmierski, R. J. Knapp, Nature 1991, 354, 82–84). Many steps have since been taken to develop this concept and reported progress range from optimization of screening conditions to the development of suitable library decoding techniques.

It has now surprisingly turned out, that enzymatic assays can be carried out in this format, i.e. inside the beads.

In the present invention the novel "in-bead" screening is based on the observation that products covalently released from polymeric beads by photolysis remain inside the beads, when they are swelled in aqueous buffer. Such compounds readily leave the beads upon washing with organic solvents. A measure for this phenomenon is given by the partition coefficient between the aqueous buffer and the organic environment provided by the bead. Thus, in aqueous buffer each bead comprises a spatially separated "micro-compartment" into which a compound can be released.

A further aspect of the present invention thus concerns a method for screening a library of hydroxamic acid derivatives for their physicochemical or biological response in a chemical or biological system, comprising:

a) obtaining a library of immobilized hydroxamic acid derivatives according to the method of the present invention,

b) releasing said hydroxamic acid derivatives by photolytic cleavage in an aqueous media,

c) adding said chemical or biological system to said library in an aqueous solution, and

d) detecting solid support, e.g. beads, showing a physiochemical or biological response, and

e) identifying hydroxamic acid derivatives immobilized on solid support,, e.g. beads, showing said physiochemical or biological response.

Step b) may be performed before step c) or step c) may be performed before step b) in the method for screening a library of hydroxamic acid derivatives for their physicochemical or biological response in a chemical or biological system.

As discussed above, the screening method may include two or more rounds of releasing compounds and testing them in different assays, i.e. different chemical or biological systems, before the identification step.

As a test system, the screening of putatively active HDAC inhibitors was demonstrated. Screening experiments were carried out using an HDAC Fluorimetric Assay/Drug Discovery Kit commercially available from Enzo Life Sciences.

For the enzyme inhibition assay, differently functionalized library beads were placed in a flat-bottomed glass dish and a buffered aqueous solution added prior to irradiation with UV-light (360 nm) to release compounds into the interior of each bead. Afterwards an aqueous buffer solution of HDAC substrate was added and the mixture left to equilibrate for some time, which resulted in absorption or intake of substrate from the aqueous solution into the organic environment inside the beads. The surrounding substrate solution was removed, followed by washing the beads once with pure buffer. Finally, buffer followed by HeLa HDAC extract solution were added and allowed to equilibrate for a sufficient time. The HDAC reaction was developed by the addition of a developer solution containing the known inhibitor TSA to simultaneously quench any further deacetylation reaction. A blue coloration of beads upon inspection of the plates under a fluorescence microscope indicates that no inhibition of HDAC activity has taken place. In contrast, beads remaining colorless indicate that HDAC activity was inhibited by the compounds released inside these beads (see Figure 5 for a graphical illustration of the in-bead HDAC assay).

To show successful extraction of substrate into the beads, beads suspended in substrate solution were isolated and surrounding aqueous buffer-solution removed by a pipette. The beads were washed twice with buffer followed by CH_3CN. HPLC-analysis of combined buffer- and CH_3CN-wash, respectively, showed no substrate in the buffer-wash, while the CH_3CN wash contained a mixture of light-released compound and substrate.

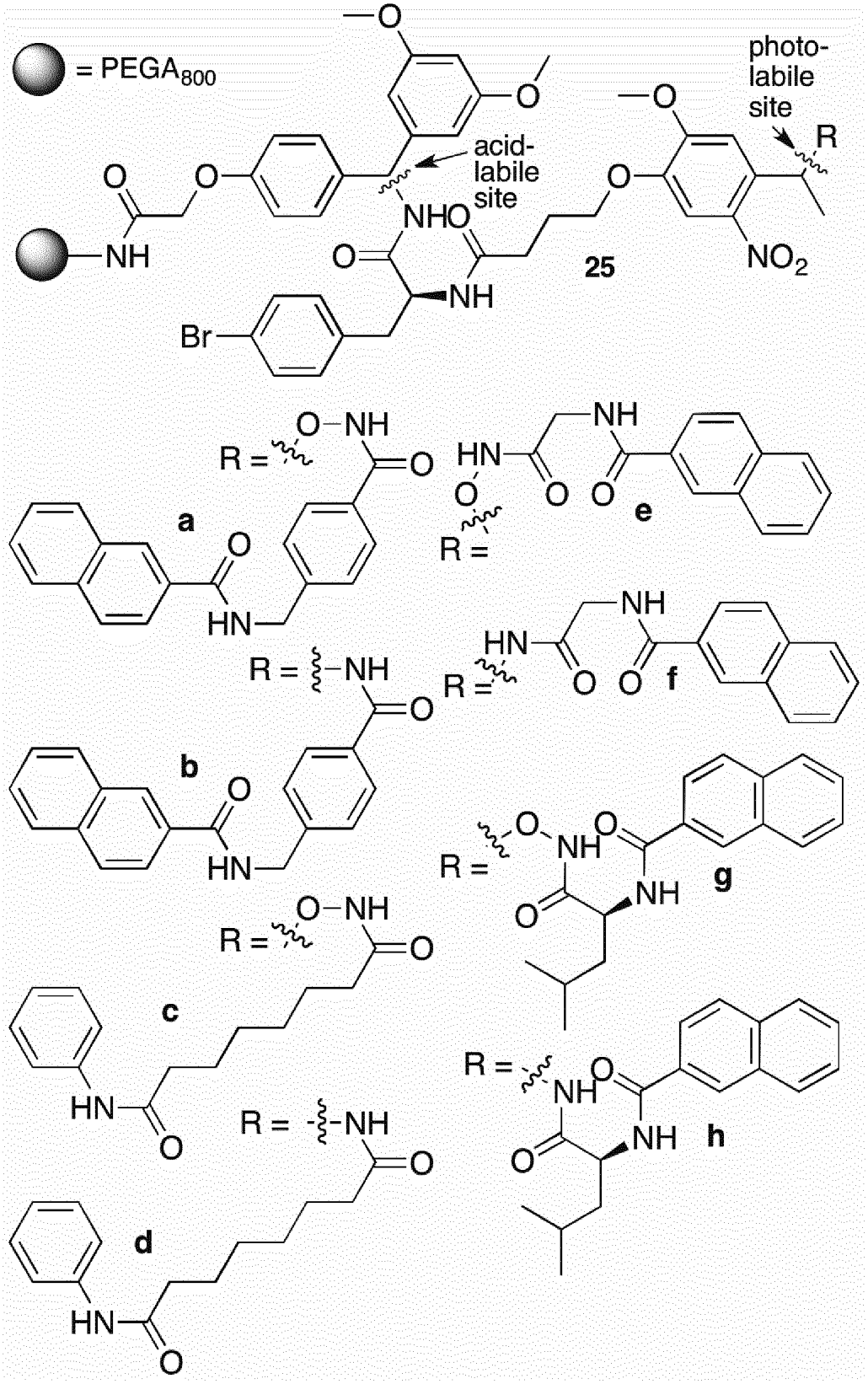
To facilitate post-screening MALDI-TOF MS sequencing of active beads, a 4-bromophenylalanine spacer was positioned between a Rink-linker functionalized solid support and the photolabile linker unit (Figure 6). Orthogonal cleavage of the acid-labile Rink linker (see Figure 6) provides a cleavage product with sufficient

mass to be out of range of low-mass noise and matrix ions typically seen in the MALDI-TOF MS analysis. Furthermore, the 4-bromophenylalanine spacer generates mass peaks with a characteristic bromine isotope pattern, so that the relevant peaks of the library products are readily identified by the presence of two peaks of equal intensity [M+Na]+ (for the ^{79}Br-capped fragments) and [M+2+Na]+ (for the corresponding ^{81}Br-capped fragments).

To further test the success of the "in-bead" technology according to the present invention, beads functionalized with SAHA (25c), an approved drug and known inhibitor of histone deacetylases, were tested in the assay. Inspection of the beads under a fluorescence microscope showed pronounced inhibition of HDAC activity in the presence of SAHA (Figure 7). Significantly, no active beads were detected when the photolysis step was excluded and immediately, a bright blue coloration of the beads was observed upon inspection of the plates under a fluorescence microscope. Furthermore, beads functionalized with a ligand without HDAC-inhibitor activity (25d) resulted in no inhibition of HDAC activity and accordingly no quenching of fluorescence was observed.

The colourless beads isolated from the assay were washed with aqueous buffer and CH_3CN to remove assay components before manually transferring one bead to a MALDI target. The active bead was swollen in TFA/CH_2Cl_2 on the MALDI target and left to react before being subjected to MALDI-TOF MS analysis, which showed the expected mass of the H_2N-(4Br)Phe-PLL-SAHA-fragment.

To validate the "in-bead" technology as a useful screening technique for bead-based libraries, a small bead-based library (25a-h), including beads functionalized with SAHA (25c), was screened. A photolysis time of 2 min produced approximately 20% beads of greater than 50% inhibition (Figure 8). Shortening the time to 0.5 min decreased the number of beads with greater than 50% inhibition to 10% (Figure 9). Evidently, the photolysis time affects the concentration of the inhibitor inside the bead. With this "in-bead" assay approach it is possible to control the concentration of ligand present in the assay, i.e. carry out dose-response experiments, and distinguish high- and low-affinity ligands.



The colorless beads detected in the assay were manually picked with a pipette and transferred to individual analysis tubes. Analysis of several of the colorless beads

revealed that all of those beads contained SAHA. Isolation and analysis of colored beads showed any of the other compounds 25a-b, d-h. These results demonstrate the value of this simple "in-bead" screening strategy for the identification of enzyme inhibitors.

Hydroxamic acids are strongly metal binding compounds and many metal-dependent biological systems may be probed with these compounds.

Based on the foregoing results, a dose-response assay was performed by simply varying the light exposure time. SAHA-functionalized beads (25c) were illuminated for 5 s, 1 min and 5 min, respectively, before being subjected to the "in-bead" HDAC assay. Inspection of the beads under the fluorescence microscope showed lower fluorescence intensity of beads with higher inhibitor concentrations (longer photolysis time) (see Figure 10). These results can again be explained by the fact that a shorter photolysis time provides beads with lower concentration of inhibitor and diminished HDAC inhibition. These results clearly demonstrate the possibility of performing a dose-response assay by simply varying the light exposure time. This is a unique advantage offered by the "in-bead" technology compared with other bead-based techniques, which merely points to qualitative binding affinities.

Since each bead (60-180pmol per PEGA bead) contains a sufficient amount of compound for more than one assay, a two-tiered release strategy may be incorporated. After identifying and isolating active beads in a primary screening assay, a second photolytic release of compounds from active beads for a dose-response assay may verify the activity observed in the primary screening, or indicate another biological activity. Furthermore each bead contains sufficient amount of compound for a post-screening hit identification.

The "in-bead" screening technology provides a rapid, convenient, and efficient primary screening tool for bead-based combinatorial libraries. Regarding the ease of this method as a primary screening tool, the approach is relatively rapid in that a library can be screened in less than 1 h. Another significant advantage of this method is the low cost of the screening format in that it does not rely on costly robotics or automation instruments and only uses small amounts of biological and chemical reagents. The screening results of the HDAC inhibitor library show that this screening method is capable of providing and identifying high-affinity inhibitors

from combinatorial bead-based libraries. Rapid and unambiguous sequencing of selected beads by MALDI-TOF MS may be facilitated by a combined acid- and photolabile cleavage construct. In addition to the identification of enzyme inhibitors, the described "in-bead" technology is a generally applicable method for evaluating other biological targets by adaptation to many other chemical or biological assay systems.

The present invention will be illustrated in the following non-limiting examples.

EXAMPLES

Example 1

Ethyl 4-(4-Acetyl-2-methoxyphenoxy)butanoate (1b).

To a solution of acetovanillone **1** (35.5 g, 0.21 mol; Sigma Aldrich, Denmark: W508454-1KG) in DMF (120 mL) was added K_2CO_3 (44.3 g, 0.32 mol) and ethyl 4-bromobutyrate (31.0 mL, 0.21 mol). The mixture was stirred for 16 hours at room temperature (rt), then heated for 3 hours at 50 °C. After filtration the solution was partitioned between EtOAc and H_2O. The organic phase was washed several times with H_2O to remove the DMF. The organic phase was dried ($MgSO_4$), filtered and the solvent was removed by rotary evaporation to afford 58.9 g of **1b** (quant.) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.23 (t, *J* = 7.3 Hz, 3 H), 2.17 (pentet, *J* = 7.3 Hz, 2 H), 2.52 (t, *J* = 7.3 Hz, 2 H), 2.53 (s, 3 H), 3.88 (s, 3 H), 3.87-4.17 (m, 4 H), 6.87 (d, J = 8.4 Hz, 1 H), 7.47-7.53 (m, 2 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.4, 24.5, 26.5, 30.8, 56.2, 60.7, 68.0, 110.6, 111.4, 123.4, 130.7, 149.5, 152.8, 173.3,196.7; UPLC/MS (ESI) *m/z* 281.3 $[MH]^+$.

Ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate (2).

A solution of ketoester **1b** (10.0 g, 35.7 mmol) in 30 mL acetic anhydride was slowly added to a solution of 70% HNO_3 (200 mL) and acetic anhydride (40 mL) at 0 °C. After stirring for 3 h the reaction mixture was poured into ice-cooled water. The precipitate was immediately collected by filtration (we found that leaving the mixture for a longer time reduced the yield due to hydrolysis of the ester). The precipitate was washed extensively with water before being dried under vacuum to afford 10.8 g of **2** (82%) as a pale yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.23 (t, *J* = 7.3 Hz, 3 H), 2,10 (pentet, *J* = 7.3 Hz, 2 H), 2.50 (t, J = 7.1 Hz, 2 H), 2.51 (s, 3 H), 3.83 (s, 3 H), 4.03 (t, *J* = 7.3 Hz, 2 H), 4.10 (q, J = 7.1 Hz, 2 H), 6.95 (s, 1 H), 7.57 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.3, 24.3, 28.2, 30.4, 55.7, 59.6, 68.4, 108.6, 110.7, 132.8, 138.2, 148.8, 154.3, 173.2, 198.7; UPLC/MS (ESI) *m/z* 326.3 $[MH]^+$.

Ethyl 4-(4-(1-hydroxyethyl) -2-methoxy-5-nitrophenoxy)butanoate (3).

To a solution of **2** (4.00 g, 12.3 mmol) in 300 mL MeOH at 0 °C was slowly added $NaBH_4$ (1.2 g; 31.7 mmol) in portions. After end addition the mixture was allowed to reach rt. The reaction was complete after 3 h (as judged by TLC). The reaction was quenched by addition of 200 mL of sat. NH_4Cl (aq). The reaction was extracted with EtOAc (400 mL), washed with water (2 × 300 mL) and saturated brine (300 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed by rotary evaporation to give 4.0 g of **3** (quant.) as a pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.20 (t, *J* = 7.2 Hz, 3 H), 1.48 (d, *J* = 7.0 Hz, 3 H), 2.10 (pentet, *J* = 7.0 Hz, 2 H), 2.47 (t, *J* = 7.1 Hz, 2 H), 3.90 (s, 3 H), 4.03 (t, *J* = 7.0 Hz, 2 H), 4.08 (q, *J* = 7.1 Hz, 2H), 5.48 (q, *J* = 7.0 Hz, 1 H), 7.23 (s, 1 H), 7.50 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.9, 23.9, 27.8, 30.0, 56.0, 61.6, 68.1, 108.5, 109.8, 137.8, 138.5, 147.7, 153.8, 174.6; UPLC/MS (ESI) *m/z* 328.4 $[MH]^+$, 310.3 $[M - OH]^+$.

Ethyl 4-(4-(1-chloroethyl)-2-methoxy-5-nitrophenoxy)butanoate (4):

To a solution of **3** (4.0 g, 12.3 mmol) in 60 mL CH_2Cl_2 at 0 °C was added thionyl chloride (20 mL). The reaction was allowed to reach rt. The reaction was complete after 2 h (as judged by TLC). The reaction was evaporated to dryness and co-evaporated several times with toluene (3 × 20 mL). The residue was passed through a short plug of silica using EtOAc/heptane (1:1) as the eluent. Evaporation of the solvent gave 3.6 g of **4** (79%) as a pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.20 (t, J = 7.2 Hz, 3H), 1.80 (d, *J* = 7.1 Hz, 3 H), 2.10 (pentet, *J* = 7.0 Hz, 2 H), 2.47 (t, *J* = 7.1 Hz, 2 H), 3.92 (s, 3 H), 4.03-4.13 (m, 4 H), 5.86 (q, *J* = 7.1 Hz, 1 H), 7.23 (s, 1 H), 7.43 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.5, 24.4, 27.6, 30.8, 54.0, 56.6, 60.8, 68.5, 108.7, 110.6, 133.0, 140.0, 147.8, 154.0, 173.1; UPLC/MS (ESI) *m/z* 346.4 $[MH]^+$, 310.3 $[M - Cl]^+$.

Ethyl 4-(4-(1-((1,3-dioxoisoindolin-2-yl)oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (5)

Ethyl 4-(4-(1-chloroethyl) -2-methoxy-5-nitrophenoxy)butanoate (**4**) (2.0 g, 5.7 mmol) and *N*-hydroxyphthalimide (1.1 g, 6.8 mmol) were dissolved in DMF (40 ml) and the reaction mixture was heated to 60 °C for 12 hours. Upon cooling to rt the reaction was quenched by addition of water (100 ml). The mixture was extracted with EtOAc (200 ml) and the organic phase was washed with water (3 × 150 mL) and brine (150 ml), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was passed through a short plug of silica using EtOAc/heptane (1:1) as the eluent to yield 2.6 g of **5** (95%) as a yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.2 (t, *J* = 7.1 Hz, 3 H), 1.5 (d, *J* = 7.1 Hz, 3 H), 2.12 (pentet, *J* = 7.0 Hz, 2 H), 2.47 (t, *J* = 7.1 Hz, 2 H), 3.91 (s, 3 H), 4.05 (t, *J* = 7.0 Hz, 2 H), 4.10 (q, *J* = 7.0 Hz, 2 H), 4.99 (q, *J* = 7.0 Hz, 1 H), 7.20 (s, 1 H), 7.57 (s, 1 H), 7.88 (m, 4 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.4, 22.4, 24.4, 30.8, 56.7, 68.5, 109.3, 109.4, 123.7, 132.0, 132.2, 132.4, 140.0, 147.6, 154.4, 161.0, 173.0; UPLC/MS (ESI) *m/z* 473.2 $[MH]^+$.

Ethyl 4-(4-(1-(aminooxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (6)

Ethyl 4-(4-(1-((1,3-dioxoisoindolin-2-yl)oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (**5**) (2.6 g, 5.5 mmol) was dissolved in EtOH (30 ml). Hydrazine monohydrate (0.5 mL, 11.0 mmol) were added and the reaction mixture was refluxed for 2 hours. The resulting white precipitate was removed by filtration and the filtrate was concentrated *in vacuo* to give 1.9 g of **6** (quant.) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.2 (t, *J* = 7.1 Hz, 3 H), 1.5 (d, *J* = 7.1 Hz, 3 H), 2.01 (bs, 2H), 2.14 (pentet, *J* = 7.0 Hz, 2 H), 2.45 (t, *J* = 7.1 Hz, 2 H), 3.91 (s, 3 H), 4.05 (t, *J* = 7.0 Hz, 2 H), 4.10 (q, *J* = 7.0 Hz, 2 H), 4.99 (q, *J* = 7.0 Hz, 1 H), 7.28 (s, 1 H), 7.62 (s, 1 H); UPLC/MS (ESI) *m/z* 343.3 $[MH]^+$.

Ethyl 4-(4-(1-(((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (7)

Ethyl 4-(4-(1-(aminooxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (**6**) (1.9 g, 5.5 mmol) was dissolved in dioxane (10mL) and 10% aq. Na_2CO_3 (20mL) was added. The reaction mixture was cooled to 0 °C. A solution of Fmoc-Cl (1.6g; 6.0 mmol) in dioxane (10 mL) was slowly added. The reaction mixture was allowed to reach rt and stirred for 2h. The resulting yellow precipitate was isolated by filtration and washed several times with water. The product was lyophilized to give 2.9 g of the Fmoc-protected hydroxylamine-ethylester **7** (92%) as a yellow solid.
^{1}H-NMR (300MHz, $CDCl_3$): δ = 1.3 (t, *J* = 7.1 Hz, 3 H), 1.5 (d, *J* = 7.1 Hz, 3 H), 2.15 (pentet, *J* = 7.0 Hz, 2 H), 2.48 (t, *J* = 7.1 Hz, 2 H), 3.93 (s, 3 H), 4.13 (t, *J* = 7.0 Hz, 2 H), 4.10 (q, *J* = 7.0 Hz, 2 H), 4.78 (q, *J* = 7.0 Hz, 1 H), 7.28 (s, 1 H), 7.33 (m, 4H), 7.55 (d, 2H), 7.62 (s, 1 H); 7.88 (d, 2H), 8.01 (bs, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.4, 22.4, 24.4, 30.8, 47.2, 56.7, 61.3, 67.3, 68.5, 75.5, 109.4, 120.5, 123.7, 124.2, 126.3, 126.8, 130.0, 143.4, 149.0, 153.1, 154.4, 155.2, 156.3, 173.0; UPLC/MS (ESI) *m/z* 565.2 $[MH]^+$.

4-(4-(1-(((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (8)

Ethyl 4-(4-(1-(((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (**7**) (2.9 g, 5.1 mmol) was dissolved in a mixture of dioxane and $KHPO_4$-buffer (5:1, 50 mL) and Novozyme 435 (2 g) was added. The reaction mixture was shaken for 5 days and filtered over a plug of celite to give 2.7 g of Fmoc-protected hydroxylamine-functionalized carboxylic acid linker **8** (quant.) as a yellow solid.
^{1}H-NMR (300MHz, $CDCl_3$): δ = 1.5 (d, *J* = 7.1 Hz, 3 H), 2.15 (pentet, *J* = 7.0 Hz, 2 H), 2.48 (t, *J* = 7.1 Hz, 2 H), 3.93 (s, 3 H), 4.13 (t, *J* = 7.0 Hz, 2 H), 4.78 (q, *J* = 7.0 Hz, 1 H), 7.28 (s, 1 H), 7.33 (m, 4H), 7.55 (d, 2H), 7.62 (s, 1 H); 7.88 (d, 2H), 8.01 (bs, 1H), 10.97 (bs, 1H); UPLC/MS (ESI) *m/z* 537.4 $[MH]^+$.

Example 2

Solid-Phase Synthesis

Attachment of Fmoc-Rink linker to amino functionalized PEGA$_{800}$ beads

Fmoc-Rink linker (3 eq.), NEM (4 equiv.) and TBTU (2.88 equiv.) were mixed in DMF, and shaken for 5 min at rt. The solution was then added to amino-functionalized $PEGA_{800}$ beads pre-swelled in DMF and allowed to react for 2 hours, followed by washing with DMF (× 6). Full conversion was judged by conventional Kaiser test.

Removal of the Fmoc protecting group was accomplished with 20% piperidine in DMF for 5 min. After washing twice with DMF, the deprotection procedure was repeated with a reaction time of 30 min. The solid support was finally washed with DMF (× 8).

Attachment of Fmoc-4-bromophenylalanine to Rink linker-functionalized $PEGA_{800}$ beads

Fmoc-4-bromophenylalanine (3 equiv.) was dissolved in DMF, and NEM (4 equiv.) followed by TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to the Rink-functionalized beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The solid support was washed with DMF (× 6). Full conversion was judged by conventional Kaiser test.

Fmoc deprotection was accomplished as noted above before. The solid support was finally washed with DMF (× 8), MeOH (× 6), CH_2Cl_2 (× 6) before being lyophilized.

Attachment of 4-(4-(1-(((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid to 4-bromophenylalanine-Rink linker-functionalized $PEGA_{800}$ beads

4-(4-(1-(((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (**8**) (3 equiv.) was dissolved in DMF, and NEM (4 equiv.) followed by TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to (4-Br)Phe-Rink linker-functionalized $PEGA_{800}$ beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The solid support was washed with DMF (× 6), MeOH (× 6) and CH_2Cl_2 (× 6) before being lyophilized. Full conversion was judged by conventional Kaiser test.

Example 3

General procedure for release of compounds from Rink linker

Release of the products from the Rink linker was achieved by treatment with TFA/CH_2Cl_2 (1:1) for 2h at room temperature. The beads were filtered and washed with CH_3CN. The combined filtrates were used directly for analytical purposes.

General procedure for release of compounds from photolabile linker

Resin sample (5-30 mg) was immersed in appropriate solvent (500 µL) and irradiated for 1h. The beads were filtered and washed with CH_3CN. The combined filtrates were analyzed by RP-HPLC.

Example 4

Synthesis of hydroxamic acid-functionalized amino acid derivative containing Boc-protected α-amino group and Pbf-protected guanidinium side group (21)

Fmoc deprotection of Fmoc-protected hydroxylamine-functionalized photolabile PEGA-beads **9** was accomplished as noted above.

Fmoc-4-(aminomethyl)benzoic acid (3 equiv.) was dissolved in DMF, and NEM (4 equiv.) followed by TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to the Fmoc-deprotected hydroxylamine-functionalized photolabile PEGA-beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The solid support was washed with DMF (× 8). Full conversion was judged by conventional Kaiser test.

Fmoc deprotection was accomplished as noted above.

Fmoc-4-(aminomethyl)benzoic acid (3 equiv.) was dissolved in DMF, and NEM (4 equiv.) followed by TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to the Fmoc-deprotected hydroxylamine-functionalized photolabile PEGA-beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The beads were washed with DMF (× 8). Full conversion was judged by conventional Kaiser test.

Fmoc deprotection was accomplished as noted above.

Boc-Arg(Pbf)-OH (3 equiv.) was dissolved in DMF, and NEM (4 equiv.) followed by TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to the beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The beads were washed with DMF (× 6), MeOH (× 6) and CH_2Cl_2 (× 6) before being lyophilized. Full conversion was judged by conventional Kaiser test.

Bead sample (30 mg) was immersed in HFIP (500 µL) and irradiated for 30min. The beads were filtered and washed with CH_3CN. The combined filtrates were analyzed by RP-HPLC, showing release of **21** in >95% purity. UPLC/MS (ESI) *m/z* 676.7 $[MH]^+$.

Example 5

Synthesis of hydroxamic acid-functionalized Doxorubicin derivative (24)

Fmoc deprotection of Fmoc-protected hydroxylamine-functionalized photolabile PEGA-beads **9** was accomplished as noted above.

Mono-*tert*-butyl malonate (3 equiv.) was dissolved in DMF, and NEM (4 equiv.) followed by TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to the Fmoc-deprotected hydroxylamine-functionalized photolabile PEGA-beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The solid support was washed with DMF (× 6), MeOH (× 6) and CH_2Cl_2 (× 6) before being lyophilized. Full conversion to **22** was judged by conventional Kaiser test.

The *tert*-butyl protecting group was removed with TFA/CH_2Cl_2 (1:1) and the carboxylic acid functionalized photolabile beads were washed with CH_2Cl_2 (× 6), MeOH (× 6) and DMF (× 6).

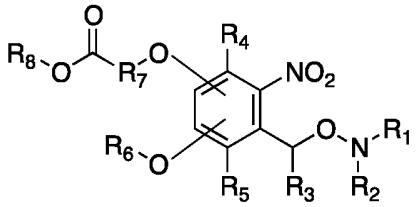
Doxorubicin (3 equiv.) was dissolved in DMF, and DIPEA (5 equiv.) followed by HATU (2.88 equiv.) were added. The mixture was shaken for 5 min at room

temperature before being added to the carboxylic acid functionalized photolabile beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The solid support was washed with DMF (× 6), MeOH (× 6), CH_2Cl_2 (× 6) before being lyophilized.

Bead sample **23** (20 mg) was immersed in HFIP (500 µL) and irradiated for 30min. The beads were filtered and washed with DMF. The combined filtrates were analyzed by RP-HPLC.and showed release of **24** in >95% purity. UPLC/MS (ESI) *m/z* 645.7 $[MH]^+$.

CLAIMS

1. A compound with the general formula I:



wherein R_1 and R_2 are the same or different and represent hydrogen, C_1-C_8 alkyl, aryl, heteroaryl, C_1-C_8 carboxyalkyl, carboxyaryl or arylalkyl,

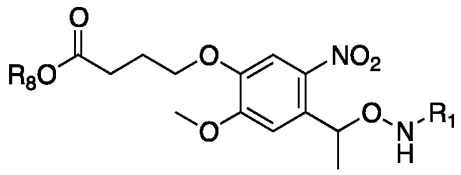
R_3 is hydrogen, C_1-C_8 alkyl, phenyl or mono- or multiply-substituted phenyl, wherein the substitutions are the same or different and represent hydrogen, C_1-C_8 alkyl, C_1-C_8 alkoxy, halogen, nitrile or nitro,

R_4 and R_5 are the same or different and represent hydrogen, C_1-C_8 alkyl, C_1-C_8 alkoxy, aryl, heteroaryl, halogen, nitrile or nitro,

R_6 and R_7 are the same or different and represent C_1-C_8 alkyl, aryl, heteroaryl or $-(CH_2-CH_2-O)-_n$, where n is a integer from 1 to 100, and

R_8 is hydrogen, C_1-C_8 alkyl or aryl.

2. A compound according to claim 1 having the formula:



wherein R_1 represents a protective group, such as Boc or Fmoc, and R_8 is hydrogen or C_1-C_8 alkyl.

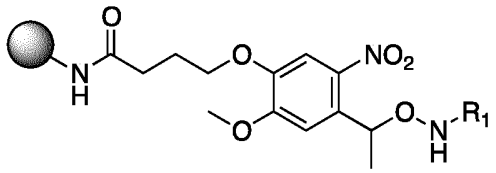
3. A method for producing a solid support comprising a hydroxylamine-functionalized photolabile linker, comprising:

a) selecting a compound according to claim 1 or claim 2,

b) removing the group R_8 from said selected compound if the group is different from hydrogen, and

c) coupling said selected compound, where R_8 is hydrogen, to a functionalized solid support.

4. A hydroxylamine-functionalized photolabile solid support having the formula:



wherein R1 is a protective group, such as Boc or Fmoc, and  represents a solid support, optionally including a spacer and/or a cleavable linker.

5. A method for synthesizing a hydroxamic acid derivative comprising:

a) coupling a compound/linker according to claim 1 or 2 to a solid support,

b) coupling a chemical group to the immobilized compound/linker, and

c) releasing said hydroxamic acid derivative from said solid support by photolytic cleavage.

6. A method for synthesizing a one–bead-one compound library of hydroxamic acid derivatives on a photolabile linker, comprising:

a) coupling a compound/linker according to claim 1 or 2 to a solid support,

b) removing the protecting group R_1,

c) coupling one or more different chemical groups to the immobilized compound/linker,

d) providing two or more different chemical groups and coupling them to the one or more chemical groups coupled to the solid support in step c),

e) repeating the coupling step d) as many times as desired/necessary to obtain the desired library.

7. A method according to claims 6, wherein the synthesis is a "split-and-mix" synthesis.

8. A library obtainable by a method according to claim 6 or claim 7.

9. A library according to claim 8, wherein the coupling between the solid support and the compound/linker comprises one or more additional cleavable linkers.

10. A library according to claim 9, wherein said one or more cleavable linkers are chemically or photolytically cleavable.

11. A method for screening a library of hydroxamic acid derivatives according to any of the claims 8-10 for their physicochemical or biological response in a chemical or biological system, comprising:

a) obtaining a library of immobilized hydroxamic acid derivatives according to the method of claim 6 or claim 7,

b) releasing said hydroxamic acid derivatives by photolytic cleavage in an aqueous media,

c) adding said chemical or biological system to said library in an aqueous media,

d) detecting solid support showing a physicochemical or biological response,

e) identifying hydroxamic acid derivatives immobilized on solid support showing said chemical or physical response.

12. A method according to claim 11 wherein step b is performed before step c or a method according to claim 11 wherein step c is performed before step b.

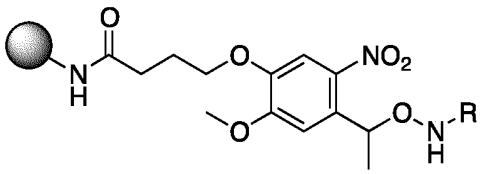
13. A method according to claim 11 or claim 12, where said detection and identification involves:

a. isolating solid support showing said physicochemical or biological response, and

b. releasing remaining hydroxamic acid derivatives from the isolated solid support by chemical or photolytic cleavage .

14. A method, a hydroxylamine-functionalized photolabile solid support or a library according to any one of the claims 3-13, wherein said solid support is a polymeric bead.

15. A hydroxylamine-functionalized compound immobilized on solid support through a photolabile linker having the formula:



wherein R is a chemical entity, and ● represents a solid support, optionally including a spacer and/or a second cleavable linker.

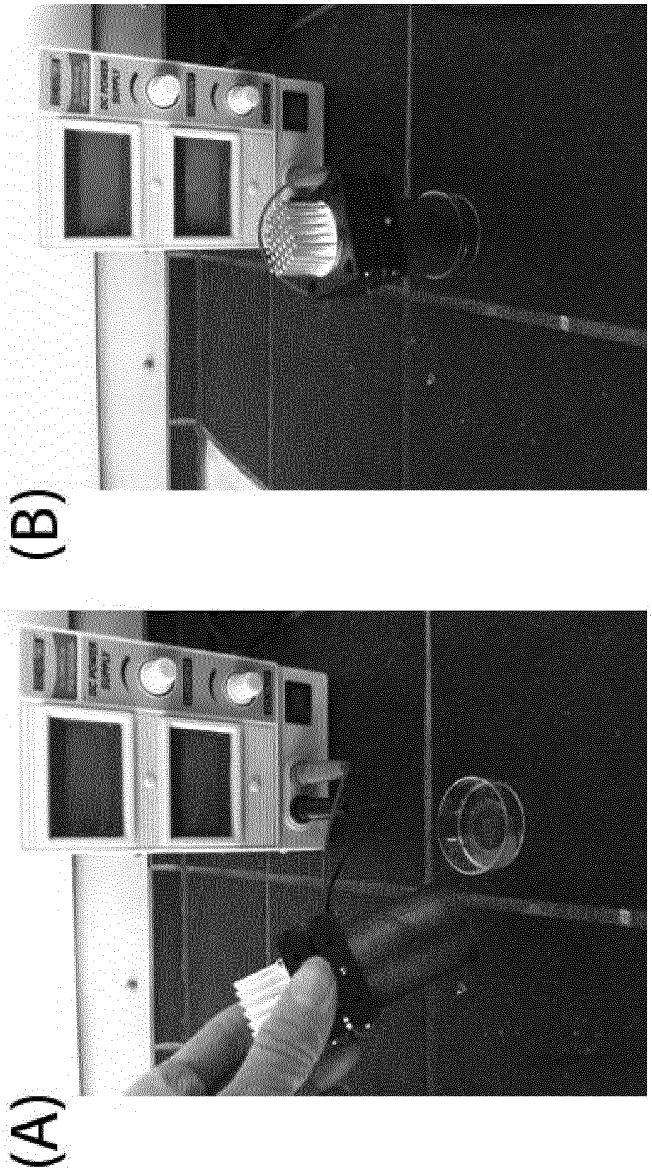


Figure 1

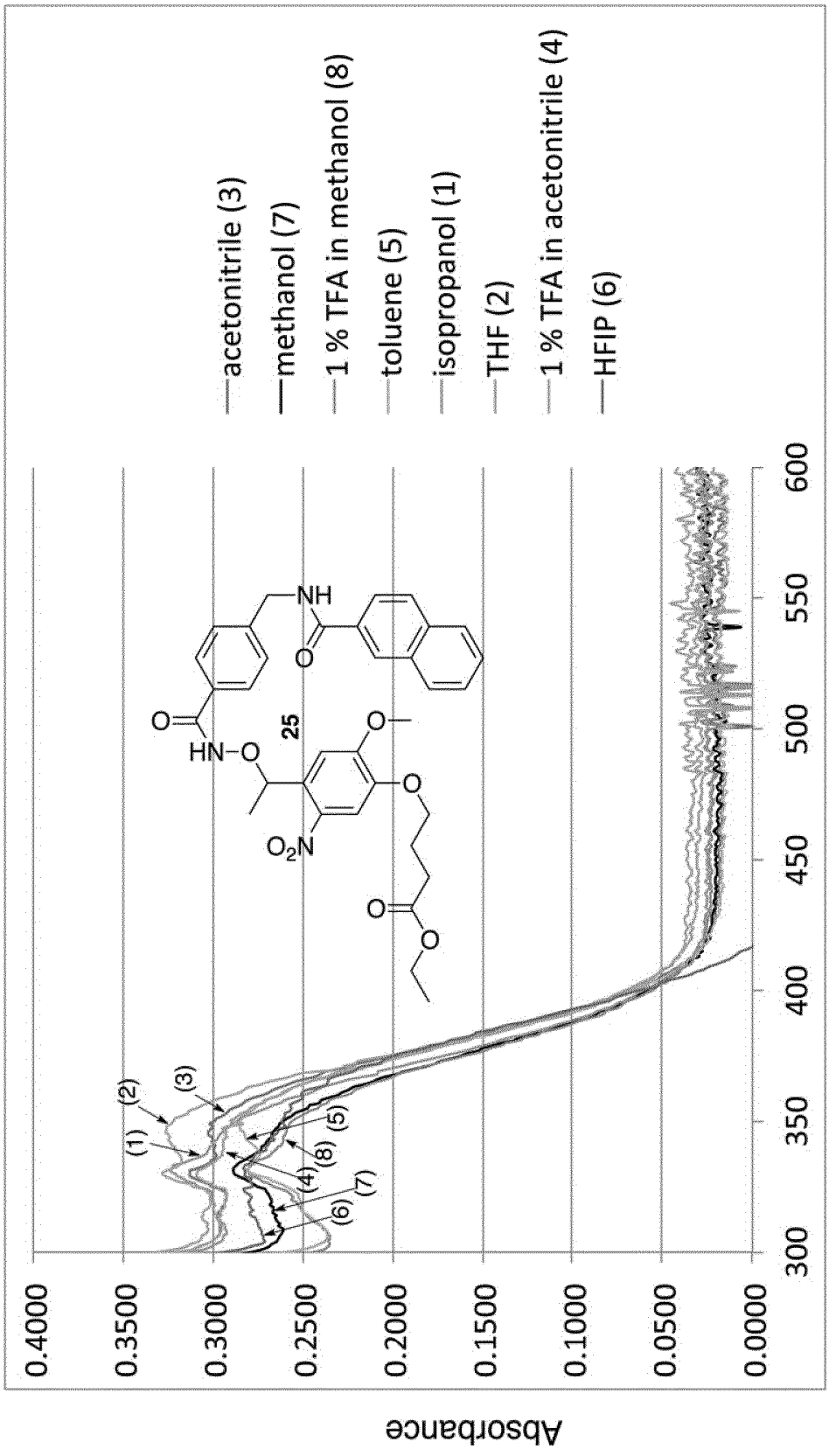

acetonitrile (3)
methanol (7)
1 % TFA in methanol (8)
toluene (5)
isopropanol (1)
THF (2)
1 % TFA in acetonitrile (4)
HFIP (6)
0.4000
0.3500
0.3000
0.2500
0.2000
0.1500
0.1000
0.0500
0.0000
Absorbance
300
350
400
450
500
550
600
wavelength (nm)
(1)
(2)
(3)
(4)
(5)
(6)
(7)
(8)
NH
O
HN–O
O
25
O
O
O_2N
O
O

Figure 2

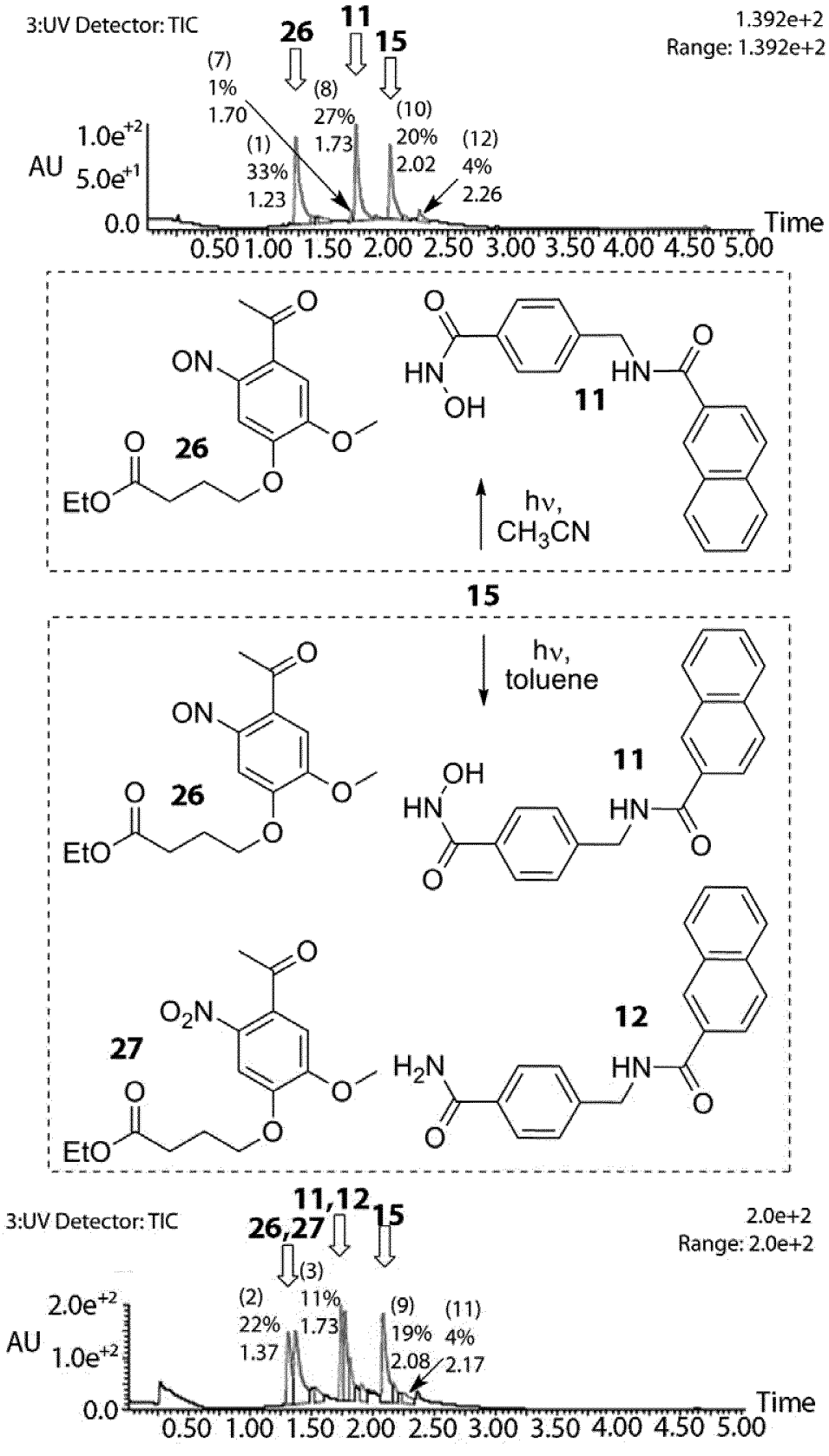

3:UV Detector: TIC
26
11
15
1.392e+2
Range: 1.392e+2
(7)
1%
1.70
(8)
27%
1.73
(10)
20%
2.02
(12)
4%
2.26
(1)
33%
1.23
AU
1.0e+2
5.0e+1
0.0
Time
0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00
ON
O
HN
OH
HN
11
26
EtO
hν,
CH3CN
15
hν,
toluene
O2N
27
12
H2N
3:UV Detector: TIC
11,12
26,27
15
2.0e+2
Range: 2.0e+2
(3)
(2)
22%
1.37
11%
1.73
(9)
19%
2.08
(11)
4%
2.17
2.0e+2
1.0e+2
0.0

Figure 3

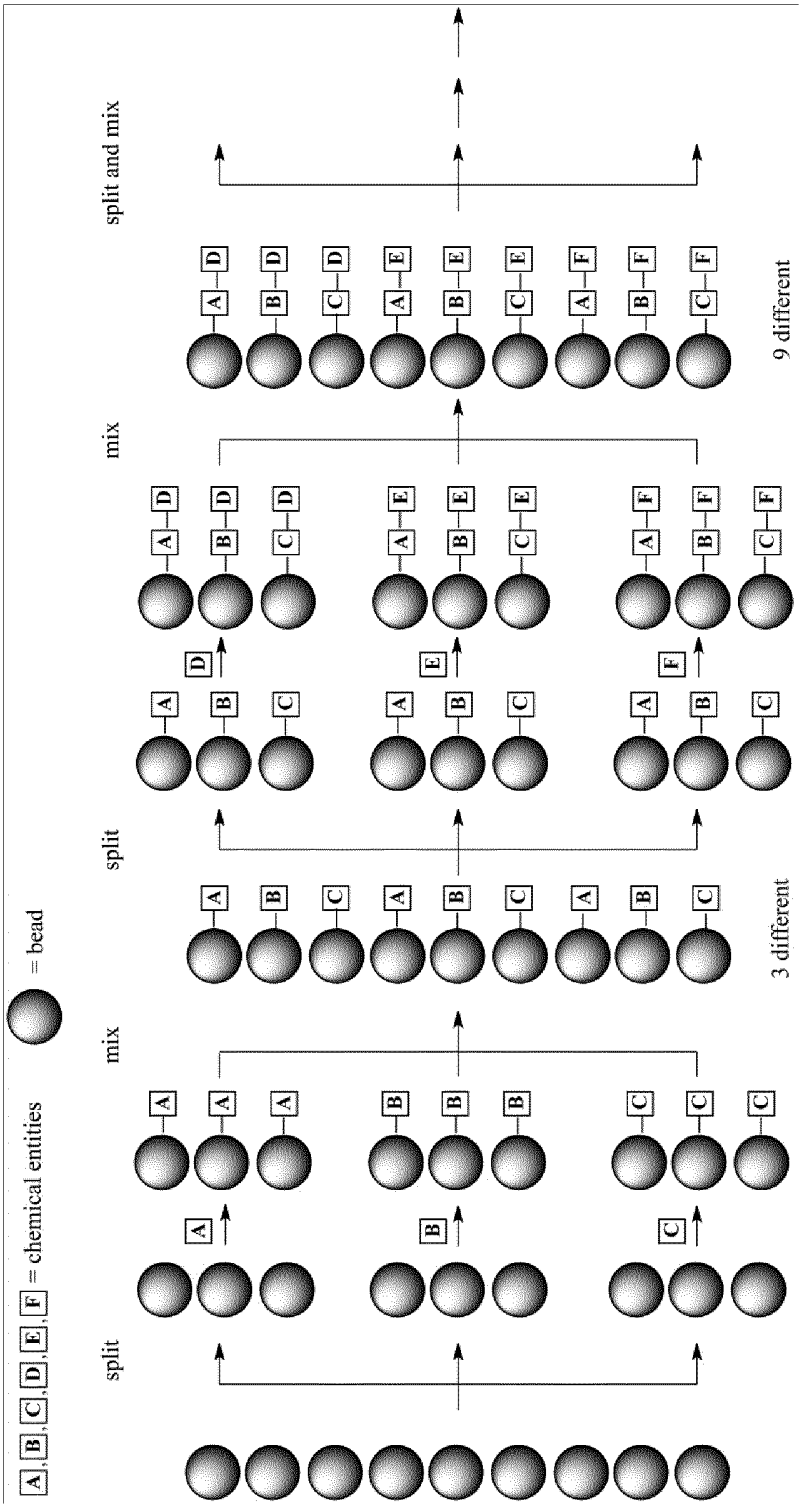

A, B, C, D, E, F = chemical entities
= bead
split
mix
split
mix
split and mix
3 different
9 different

Figure 4

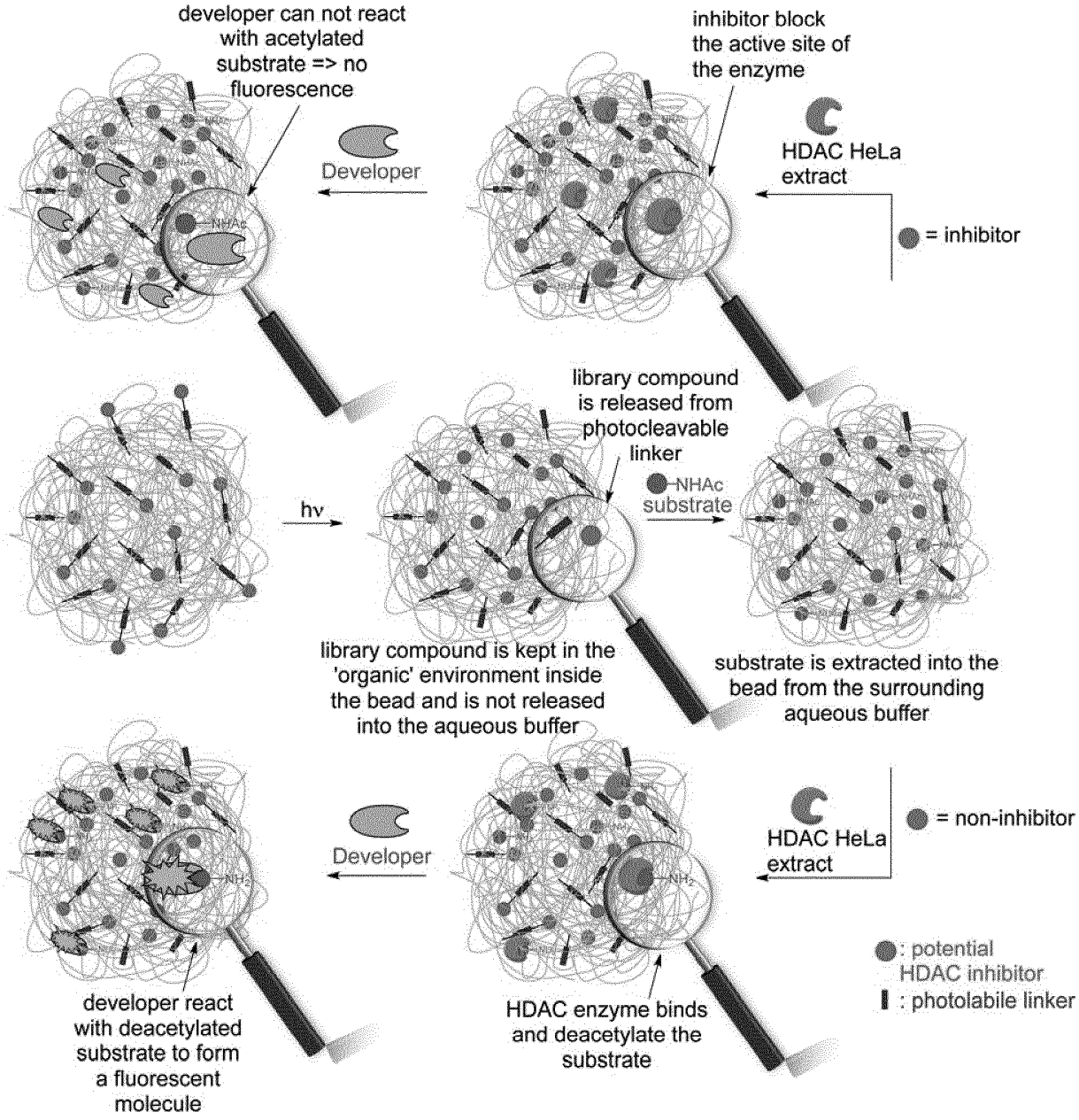

developer can not react with acetylated substrate => no fluorescence
Developer
inhibitor block the active site of the enzyme
HDAC HeLa extract
= inhibitor
library compound is released from photocleavable linker
NHAc substrate
hv
library compound is kept in the 'organic' environment inside the bead and is not released into the aqueous buffer
substrate is extracted into the bead from the surrounding aqueous buffer
Developer
HDAC HeLa extract
= non-inhibitor
: potential HDAC inhibitor
: photolabile linker
developer react with deacetylated substrate to form a fluorescent molecule
HDAC enzyme binds and deacetylate the substrate

Figure 5

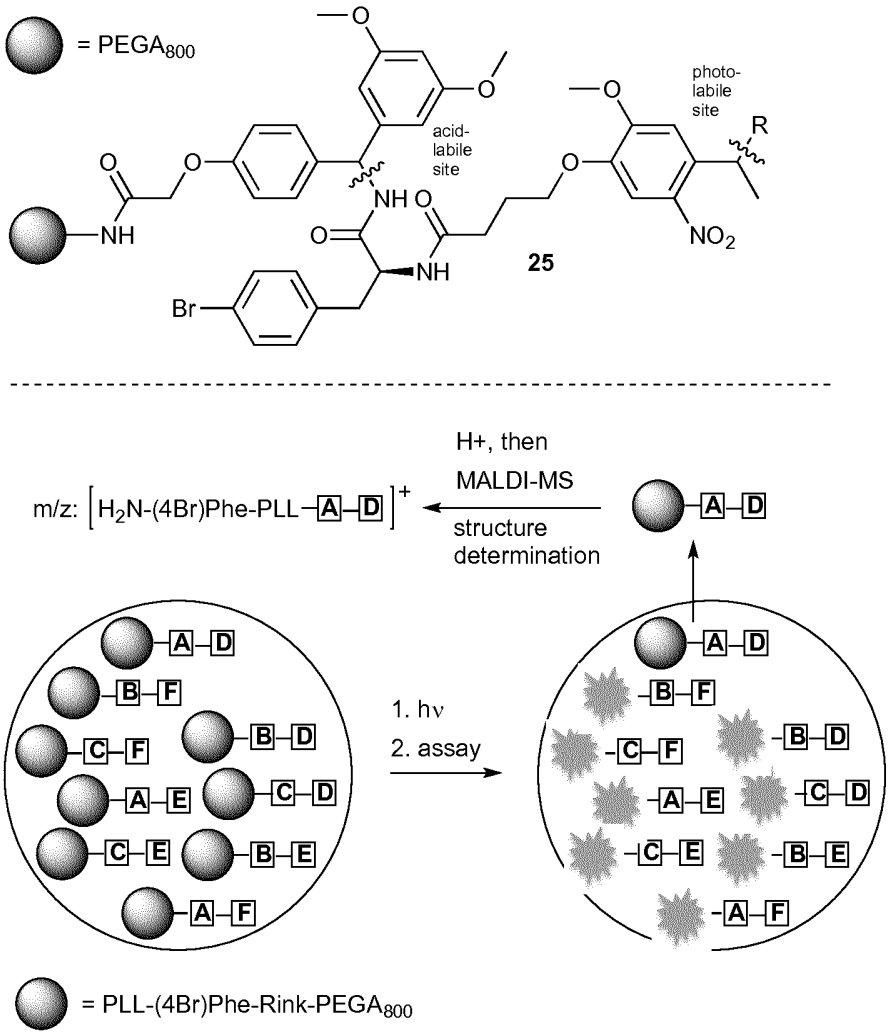

= PEGA800
acid-labile site
photo-labile site
R
NO2
NH
Br
25
H+, then
MALDI-MS
structure determination
m/z: [H2N-(4Br)Phe-PLL-A-D]+
1. hν
2. assay
= PLL-(4Br)Phe-Rink-PEGA800

Figure 6

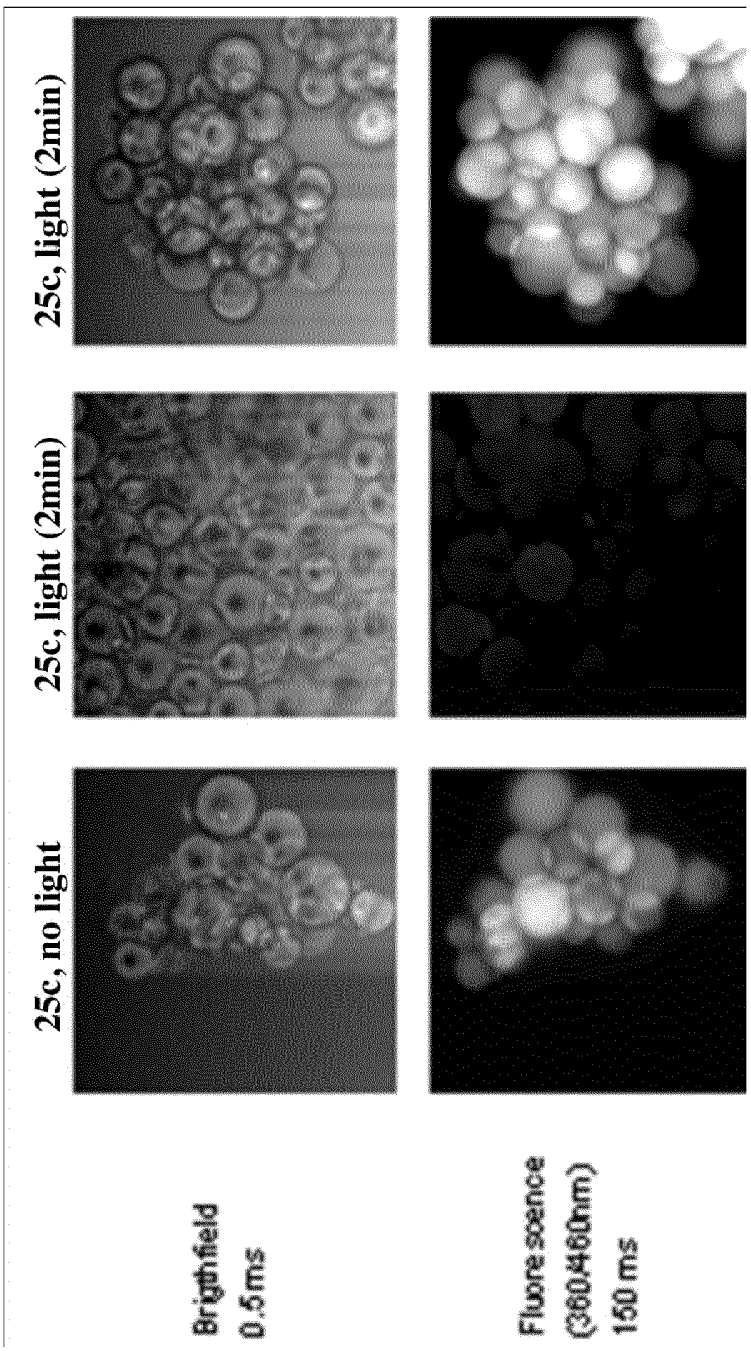

25c, light (2min)
25c, light (2min)
25c, no light
Brightfield
0.5 ms
Fluorescence
(360,460nm)
150 ms

Figure 7

5

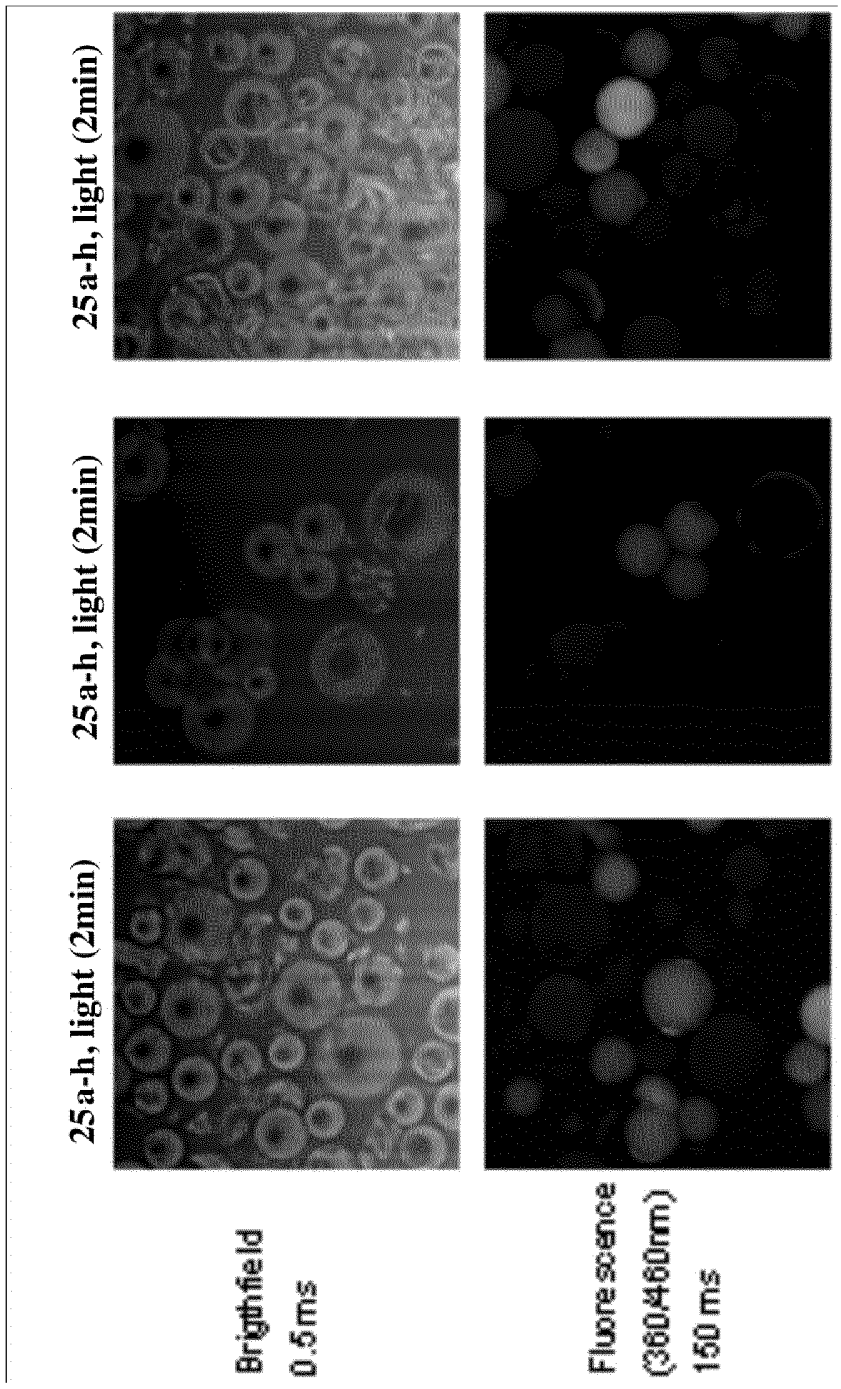

25a-h, light (2min)
25a-h, light (2min)
25a-h, light (2min)
Brightfield
0.5ms
Fluorescence
(360,460nm)
150 ms

Figure 8

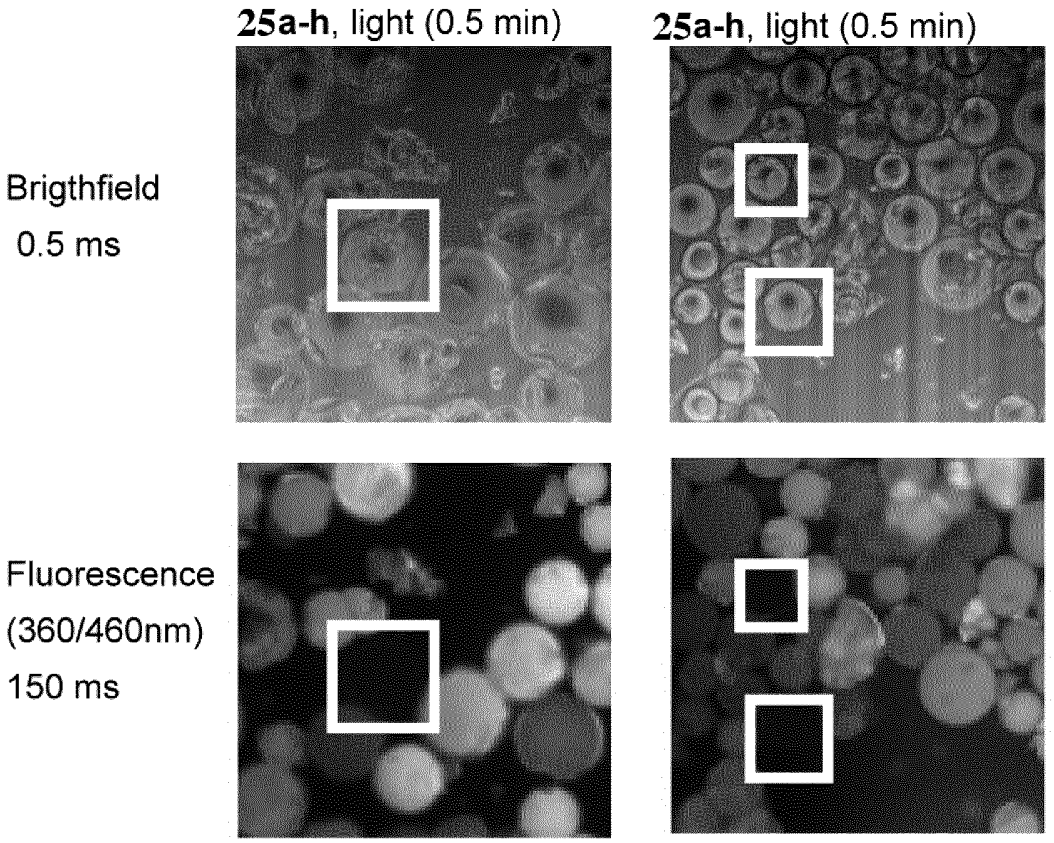

25a-h, light (0.5 min)
25a-h, light (0.5 min)
Brigthfield
0.5 ms
Fluorescence
(360/460nm)
150 ms

Figure 9

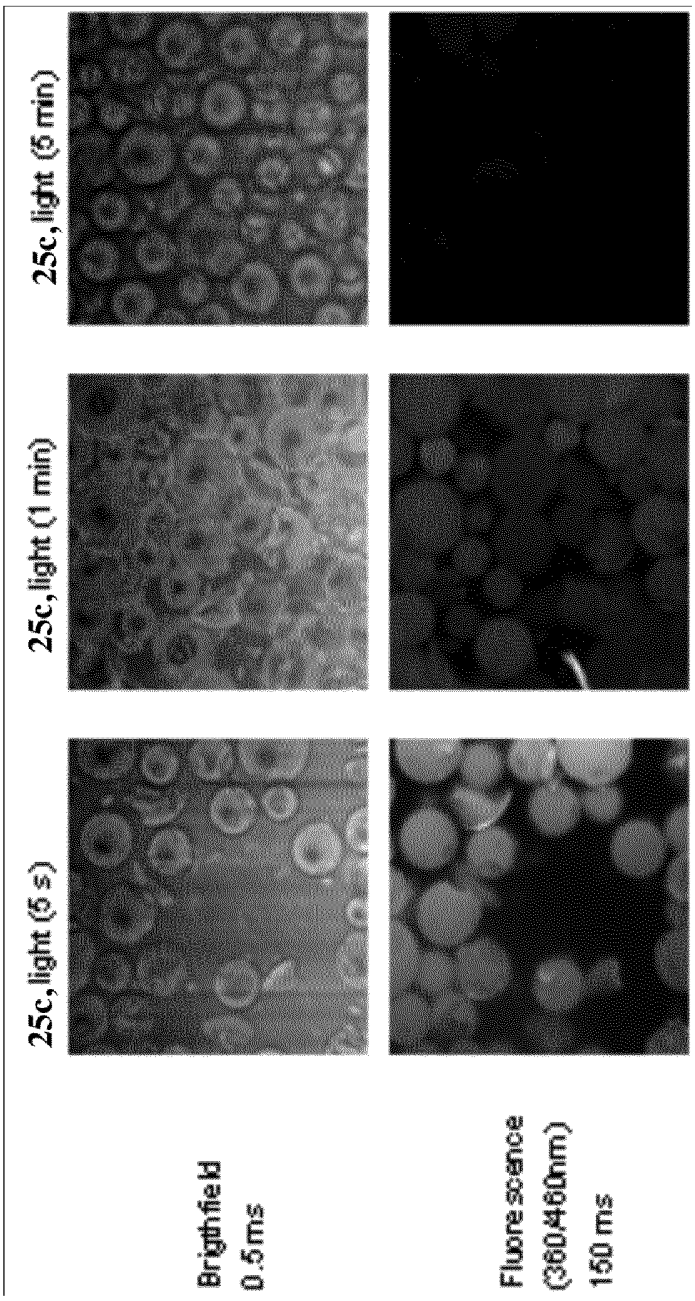

25c, light (5 s)
25c, light (1 min)
25c, light (5 min)
Brightfield
0.5 ms
Fluorescence
(360,460nm)
150 ms

Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/070648

A. CLASSIFICATION OF SUBJECT MATTER
INV. C40B40/04 C40B50/14 C40B80/00 C07C235/06 C07C237/22
C07C237/42 C07C259/06 C07C259/10 C40B30/04
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C40B C07C

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/26223 A1 (BRITISH BIOTECH PHARM [GB]; FLOYD CHRISTOPHER DAVID [GB]; LEWIS CHRIST) 29 August 1996 (1996-08-29) cited in the application example H -----	4,15
A	KATRINE QVORTRUP ET AL: "A photolabile linker for the solid-phase synthesis of 4-substituted NH-1,2,3-triazoles", CHEMICAL COMMUNICATIONS, vol. 47, no. 11, 1 January 2011 (2011-01-01), pages 3278-3280, XP055021594, ISSN: 1359-7345, DOI: 10.1039/c0cc05274d cited in the application the whole document -----	1-7, 11-15
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 2012	25/01/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Österle, Carmen

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Form PCT/ISA/210 (second sheet) (April 2005)

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WHITEHOUSE D L ET AL: "An Improved Synthesis and Selective Coupling of a Hydroxy Based Photolabile Linker for Solid Phase Organic Synthesis", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 38, no. 45, 10 November 1997 (1997-11-10), pages 7851-7852, XP004097187, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(97)10133-2 cited in the application the whole document -----	1-7, 11-15
A	MAEDA TAISHI ET AL: "Potent histone deacetylase inhibitors: N-hydroxybenzamides with antitumor activities", BIOORGANIC & MEDICINAL CHEMISTRY, PERGAMON, GB, vol. 12, no. 16, 1 July 2004 (2004-07-01), pages 4351-4360, XP008122886, ISSN: 0968-0896 compounds 6a-6i -----	1-7, 11-15
A	V. KRCHNAK: "Solid-Phase Synthesis of Biologically Interesting Compounds Containing Hydroxamic Acid Moiety", MINI-REVIEWS IN MEDICINAL CHEMISTRY, vol. 6, 2006, pages 27-36, XP009157345, cited in the application the whole document -----	1-7, 11-15

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International application No.
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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 11-15

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7, 11-15

   Compound of formula (I), functionalized solid support comprising the compound of formula (I), method for synthesizing a solid support comprising compound (I), a method for synthesizing a hydroxamic acid derivative using said functionalized solid support, method for synthesizing a library using said solid support, method for screening a library comprising obtaining a library of hydroxamic acid derivatives using said solid support.

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2. claims: 8-10

   Library obtainable by a method according to claim 6 or 7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/070648

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9626223 A1	29-08-1996	AT 178618 T	15-04-1999
		DE 69602016 D1	12-05-1999
		DE 69602016 T2	02-09-1999
		EP 0811019 A1	10-12-1997
		JP H11500620 A	19-01-1999
		US 5932695 A	03-08-1999
		US 6093798 A	25-07-2000
		US 6228988 B1	08-05-2001
		WO 9626223 A1	29-08-1996