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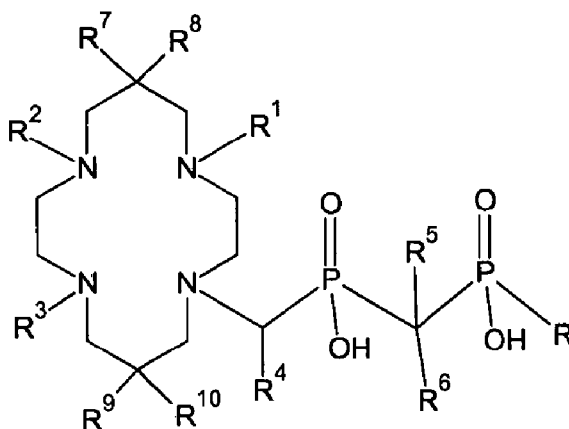
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(54) Title: CYCLAM BASED COMPOUNDS, THEIR CONJUGATES, CO-ORDINATION COMPOUNDS, PHARMACEUTICAL COMPOSITION CONTAINING THEREOF, METHOD OF PREPARATION AND USE THEREOF



(I)

(57) Abstract: Cyclam based compounds, their conjugates, co-ordination compounds, pharmaceutical composition containing thereof, method of preparation and use thereof The present invention relates to cyclam based compounds of the general formula (I), conjugates of such compounds with conjugation groups, their coordination compounds, targeting conjugates, method of preparation of cyclam based compounds, intermediate products for the preparation of cyclam based compounds, a pharmaceutical preparation containing them, and the use thereof.

Cyclam based compounds, their conjugates, co-ordination compounds, pharmaceutical composition containing thereof, method of preparation and use thereof

#### Field of Art

5

The present invention relates to cyclam based compounds, containing tetraazamacrocycle and bis-phosphorus acid, conjugates of such compounds with conjugation groups, their coordination compounds, targeting conjugate, method of preparation of cyclam based compounds, intermediate products for the preparation of cyclam based compounds,  
10 pharmaceutical preparation containing them, and the use thereof.

#### Background Art

Polyazamacrocycles with coordinating side chains form thermodynamically very stable  
15 complexes with a wide scale of metal ions that are often also highly kinetically inert. These properties thereof are very desirable for applications in medicine and molecular biology. Therefore, these ligands and complexes thereof are widely studied and their properties adapted to a particular application, as for example MRI contrast agents, carriers for metal radionuclides in diagnostic or therapeutic methods, targeted  
20 medicaments or luminescent probes.

Very powerful diagnostic methods of modern medicine are positron emission tomography (PET) or tomographic scintigraphy (single-photon emission tomography, SPECT), which are based on the application of radio-isotopes, i.e. proton-rich isotopes of elements that undergo  $\beta^+$  decay (for PET), or emitting gamma radiation (for SPECT). In PET, a  
25 collision of emitted positron with electron from the surrounding tissue leads to origination of a pair of collinear  $\gamma$ -photons (having the energy of 512 keV), which allow a precise detection of place of annihilation thus also the distribution of the radio-isotope in the body. PET requires using radio-isotopes with suitable half-life, low energy of emitted positrons and good availability (for example non-metallic radio-isotopes  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{15}\text{O}$ , or  
30 radioisotopes of metals, such as  $^{68}\text{Ga}$ ,  $^{44}\text{Sc}$ ,  $^{89}\text{Zr}$ ). Great interest was raised up by the radioisotope of copper  $^{64}\text{Cu}$  (61 %  $\beta^+$ ) thanks to its long half-life ( $\tau_{1/2}$  12.8 h) and low energy of positrons ( $E_{\text{av}}$  0.65 MeV), which leads to a high resolution in PET. Further radioisotopes of copper, positron emitting  $^{60}\text{Cu}$  ( $\tau_{1/2}$  23.7 min, 100 %  $\beta^+$ ) and  $^{61}\text{Cu}$  ( $\tau_{1/2}$  3.3 h, 100 %  $\beta^+$ ), are also used in PET, and the  $\beta^-$ -emitting  $^{67}\text{Cu}$  ( $\tau_{1/2}$  61.8 h, 100 %  $\beta^-$ ) is used

in radionuclide therapy. Radio-isotopes  $^{64}\text{Cu}$  and  $^{67}\text{Cu}$  form an interesting theranostic pair (one isotope is used for diagnostics and the second isotope for therapy).

Radioisotopes of metals mostly cannot be applied in a form of free ions because of non-specific accumulation in tissues. In order to achieve the required bio-distribution, the metal ion shall be bound in a thermodynamically stable and kinetically inert complex, and this complex is often conjugated to the targeting vector. There are various chelators for a formation of thermodynamically stable and kinetically inert complexes of metals, while the macrocyclic ligands are preferred. For the complexation of copper radioisotopes, both acyclic and macrocyclic ligands are used, for example diethylenetriaminepentaacetic acid (H<sub>5</sub>dtpa), 1,4,8,11-tetraazacyclotetradecane (cyclam), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (H<sub>4</sub>teta), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (H<sub>4</sub>dota), their monoamides and other derivatives. However, their properties are not optimal for complexation of copper(II) ions, especially because of a slow complexation, low kinetic inertness, and unsuitable redox potential shifted towards copper(I) ions, and, therefore, other ligands were studied, for example derivatives of bridged cyclam, which form kinetically very inert complexes, however, they are burdened by a very slow complexation.

Preparation of various macrocyclic ligands on the basis of cyclen (1,4,7,10-tetraazacyclododecane) or cyclam (1,4,8,11-tetraazacyclotetradecane), with a pendant group containing phosphorus or eventually arsenic, is described for example in US 2008/0312430 A1 or WO2015/038968. The document Fuzerová S., Kotek J., Císařová I., Hermann P., Binnemans K., Lukeš I., *Dalton Trans.*, **2005**, 2908–2915, describes the synthesis, the potentiometric and NMR titrations, and the crystallographic structure of a ligand on the basis of cyclam with a pendant phosphonic acid and its copper(II) complexes. The document Kotek J., Lubal P., Hermann P., Císařová I., Lukeš I., Godula T., Svobodová I., Táborský P., Havel J., *Chem. Eur. J.*, 2003, **9**, No. 1, 233–248, describes an analogous compound, which, however, contains two pendant phosphonic acids in a trans position. This publication also studies the synthesis, potentiometry, and crystallographic study of the ligand and its copper complexes. It results from the two last mentioned documents that one phosphonic acid group is coordinated to Cu<sup>II</sup> and it forms a thermodynamically stable and kinetically inert complex. The second phosphonic acid group present in the compound studied in Kotek J., Lubal P., Hermann P., Císařová I., Lukeš I., Godula T., Svobodová I., Táborský P., Havel J., *Chem. Eur. J.*, 2003, **9**, No. 1, 233–248 does not coordinate to the copper(II) ion and the thermodynamic stability of

copper(II) complexes of both ligands mentioned above is comparable. The cited compounds, however, are not bifunctional, they cannot thus be linked to any targeting vector in order to get to a specific place in the body during biodistribution. Moreover, although the substances cited form stable copper complexes, the complexation rate is not sufficient for that the resulting complex to be created quickly and, if possible, at room temperature. Thus, for the practical use in radiodiagnostics or radiotherapy, these substances are not suitable because of their non-selective biodistribution and low complexation rate.

For targeted biodistribution and dosage reduction, the conjugation of a complex with a targeting vector is used. Targeting vectors may be, for example, various peptides, antibodies and their fragments or derivatives, biotin, folic acid, cyclodextrins, dendrimers, polymers, polysaccharides.

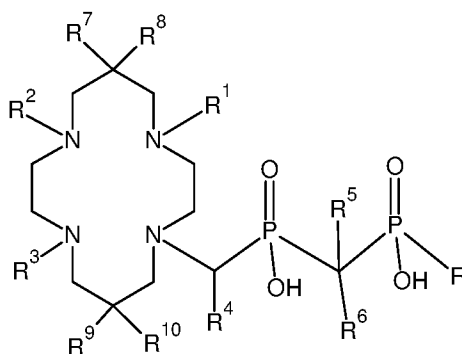
The disadvantage of the prior art is the absence of substances with high specific activity, which is a value indicating the percentage representation of the complex of the radioisotope in the total dose of the medicament. This value is closely related with the capability of ligands to form a thermodynamically stable and kinetically inert coordination compound with radionuclide, with a suitable half-life, quickly and, if possible, at room temperature, which would, at the same time, contain a targeting vector, and therefore would be suitable for applications in diagnostic (for example PET, SPECT) and therapeutic methods. The existing ligands effectively complex radionuclides at temperatures of approximately 80 °C and higher, while at room temperature their complexation is very slow, which causes, for example, loss of radioisotope complexes with a short half-life, high financial costs associated with preparation of radioisotope complexes, as well as the stress of the patient exposed to an application of high doses of the medicament in order to achieve the desired effect.

#### Disclosure of Invention

The present invention relates to compounds based on cyclam and/or bridged cyclam for therapy and diagnostics, containing a bis-phosphorus acid, which are characterized by a high specific activity. The compounds according to the present invention complex metal ions quickly and selectively, in particular  $\text{Cu}^{2+}$  ions, and they form thermodynamically stable and kinetically inert coordination compounds. The compounds according to the present invention may further form conjugates with conjugation groups and further

targeting conjugates with targeting vectors that ensure their targeted distribution in the organism.

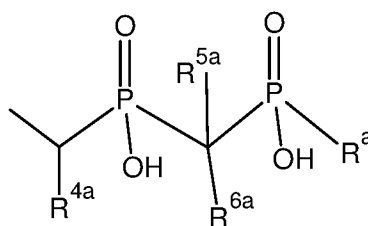
The subject of the present invention are cyclam based compounds for therapy and  
5 diagnostics of the general formula (I)



(I)

wherein

R, R<sup>a</sup>, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> are independently selected from the group comprising  
10 H, OH, (C1 to C6)alkyl, which may be linear or branched, (C3 to C6)cycloalkyl, benzyl,  
and/or R<sup>1</sup> and/or R<sup>2</sup> and/or R<sup>3</sup> is a bis-phosphorus acid of the general formula (II)



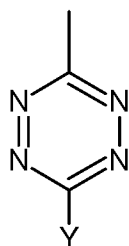
(II)

and/or R<sup>1</sup> and R<sup>3</sup> together form (C2 to C3)alkylene, which may be substituted with one or  
15 more linear or branched (C1 to C6)alkyls,

and/or R, R<sup>a</sup> are independently selected from phenyl and (C5 to C6)heterocycle,  
containing at least one heteroatom of N, S, O,

wherein the (C1 to C6)alkyl, (C3 to C6)cycloalkyl, phenyl, benzyl and (C5  
to C6) heterocycle, containing at least one heteroatom of N, S, O, may be  
20 independently substituted with one or more groups selected from COOH;  
NH<sub>2</sub>; NO<sub>2</sub>, NX<sub>2</sub>; C(O)NX<sub>2</sub>; NHX; C(O)NHX; OH; SH; -NCS; tetrazine of  
the general formula (III);

5



(III)

(C7 to C8)cycloalkenyl in *trans* configuration, which may eventually be further substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups chosen from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cycloazaocynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z;

and wherein R<sup>4</sup>, R<sup>4a</sup>, R<sup>5</sup> and R<sup>5a</sup> are independently H, (C1 to C6)alkyl, which may be linear or branched, (C3 to C6)cycloalkyl, benzyl;

wherein (C1 to C6)alkyl, (C3 to C6)cycloalkyl and benzyl may be independently substituted with one or more groups selected from COOH; NH<sub>2</sub>; NO<sub>2</sub>, NX<sub>2</sub>; C(O)NX<sub>2</sub>; NHX; C(O)NHX; OH; SH; -NCS; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans* configuration, which may eventually be further substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cycloazaocynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z;

and wherein R<sup>6</sup> and R<sup>6a</sup> is H;

and wherein R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup> and R<sup>10</sup> are independently selected from the group comprising H, (C1 to C6)alkyl, (C3 to C6)cycloalkyl, benzyl, phenyl, NO<sub>2</sub>, COOH, (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O, and/or altogether R<sup>7</sup> and R<sup>8</sup> contain (C2 to C3)alkylene or vinylene and/or altogether R<sup>9</sup> and R<sup>10</sup> contain (C2 to C3)alkylene or vinylene,

wherein (C1 to C6)alkyl, (C3 to C6)cycloalkyl, phenyl, benzyl, (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O, (C2 to C3)alkylene and vinylene may be independently substituted with a group or groups selected from COOH; NH<sub>2</sub>; NO<sub>2</sub>; NX<sub>2</sub>; C(O)NX<sub>2</sub>; SH; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans* configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclozaaoctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z,

wherein Y is H, (C1 to C6)alkyl, -CH<sub>2</sub>COOH, (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O, or heteroaryl, containing at least one heteroatom of N, S, O, preferably Y is an atom of hydrogen, methyl or pyridyl;

wherein Z is H; OH; (C1 to C6)alkyl, which may be linear or branched; (C1 to C6)hydroxyalkyl; (C1 to C6)alkoxyl; phenyl; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; or benzyl, wherein phenyl, (C5 to C6)heterocycle or benzyl may eventually be substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub> and SH, preferably the substituent is in *para* position;

and wherein X is independently H; (C1 to C6)alkyl, which may be linear or branched; (C3 to C6)cycloalkyl; phenyl and/or benzyl;

while for X, Y and Z applies, that (C1 to C6)alkyl, (C3 to C6)cycloalkyl, phenyl and/or benzyl may be independently substituted with one or more groups selected from COOH; NH<sub>2</sub>; NO<sub>2</sub>; NX<sub>2</sub>; C(O)NX<sub>2</sub>; NHX; C(O)NHX; SH; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans*

- configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cycloazaoctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z;
- 10 wherein at least one of the groups R, R<sup>a</sup>, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>4a</sup>, R<sup>5</sup>, R<sup>5a</sup>, R<sup>6</sup>, R<sup>6a</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup> a R<sup>10</sup> contains at least one of the groups -COOH; NH<sub>2</sub>; NO<sub>2</sub>, NX<sub>2</sub>, NHX; C(O)NHX; N<sub>3</sub>; SH; -NCS; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans* configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; -N<sub>3</sub>; (C2 to C6)alkynyl; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; cycloazaoctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; maleimide; phenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; benzyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; (C1 to C6)alkyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; and -P(O)(OH)Z.
- 15
- 20
- 25

In one embodiment of the invention, R<sup>1</sup> and R<sup>3</sup> together form (C2 to C3)alkylene, which may be substituted with (C1 to C6)alkyl, which may be linear or branched.

- 30 In another embodiment of the invention, R, R<sup>a</sup>, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>4a</sup>, R<sup>5</sup>, R<sup>5a</sup>, R<sup>6</sup> and R<sup>6a</sup> are independently selected from the group comprising H; (C1 to C6)alkyl, which may be linear or branched; (C3 to C6) cycloalkyl; benzyl; phenyl; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O;

and/or R<sup>1</sup> and/or R<sup>2</sup> and/or R<sup>3</sup> is the bis-phosphorus acid of the general formula (II), preferably at least one of the groups R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> is the bis-phosphorus acid of the general formula (II), most preferably R<sup>2</sup> is the bis-phosphorus acid of the general formula (II).

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In one preferred embodiment, R<sup>2</sup> is selected from the group comprising H; (C1 to C6)alkyl, which may be linear or branched; (C3 to C6)cycloalkyl; benzyl; phenyl; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; and bis-phosphorus acid of the general formula (II).

10

In another preferred embodiment, the cyclam-based compounds according to the present invention are selected from the group comprising compounds of the general formula (I), whose the substituents are represented in the following combinations:

Compound number	R	R <sup>1</sup>	R <sup>3</sup>	R <sup>2</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	R <sup>7</sup>	R <sup>8</sup>	R <sup>9</sup>	R <sup>10</sup>
1	H	H	H	H	CH <sub>3</sub>	H	H	H	H	H	H
2	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	H	H	H
3	H	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	H	H	H	H	H
4	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	H	H	H	H
5	OH	benzyl	benzyl	H	H	H	H	H	H	H	H
7	OH	H	benzyl	benzyl	H	H	H	H	H	H	H
8	<i>p</i> -nitrobenzyl	H	H	H	H	H	H	H	H	H	H
9	<i>p</i> -aminobenzyl	H	H	H	H	H	H	H	H	H	H
10	<i>p</i> -SCN-benzyl	H	H	H	H	H	H	H	H	H	H
11	<i>p</i> -azidobenzyl	H	H	H	H	H	H	H	H	H	H
12	-CH <sub>2</sub> N(Bz) <sub>2</sub>	H	H	H	H	H	H	H	H	H	H
13	-CH <sub>2</sub> NH <sub>2</sub>	H	H	H	H	H	H	H	H	H	H
14	-(CH <sub>2</sub> ) <sub>2</sub> COOH	H	H	H	H	H	H	H	H	H	H
15	H	H	H	H	H	-(CH <sub>2</sub> ) <sub>4</sub> COOH	H	H	H	H	H







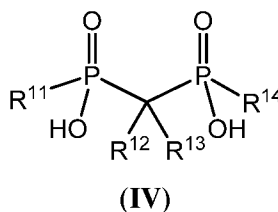
The subject of the present invention is also a conjugate of the cyclam-based compound according to the present invention and of at least one conjugation group, which is covalently bound to the cyclam-based compound, and which is selected from the group containing OH, -COOH; NH<sub>2</sub>; NO<sub>2</sub>, NX<sub>2</sub>, NHX; C(O)NHX; N<sub>3</sub>; SH; -NCS; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans* configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX; C(O)NHX; N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX; C(O)NHX; N<sub>3</sub> and SH; -N<sub>3</sub>; (C2 to C6)alkynyl; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX; C(O)NHX; N<sub>3</sub> and SH; cycloazaotynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, (C1 to C6)alkyl, (C6)aryl, N<sub>3</sub> and SH; maleimide; and -P(O)(OH)Z; wherein the conjugate may further contain a spacer between the cyclam-based compound and the conjugation group and/or between two conjugation groups, the spacer selected from the group containing (C1 to C6)*n*-alkyl, eventually substituted with C=O and/or -NH- group; phenylene; amino-acids chains with the length of 1 to 5 amino-acids; polyethylene glycols of 1 to 10 monomeric units.

The subject of the present invention are also coordination compounds of the cyclam based compounds of the general formula (I) or the conjugates according to the present invention, with metal cations selected from the group consisting of Cu<sup>2+</sup>, Bi<sup>3+</sup>, lanthanide cations, Sc<sup>3+</sup>, Y<sup>3+</sup>, Pb<sup>2+</sup>, Zr<sup>4+</sup>, Ac<sup>3+</sup>, Mn<sup>2+</sup> and Mn<sup>3+</sup>, preferably lanthanide cations, Bi<sup>3+</sup>, Y<sup>3+</sup> a Cu<sup>2+</sup>, more preferably Lu<sup>3+</sup>, Bi<sup>3+</sup>, Y<sup>3+</sup> and Cu<sup>2+</sup>. Metal cations may preferably be radioisotopes of metals, especially radioisotopes of copper, lutetium, bismuth, and yttrium.

The subject of the present invention is also a targeting conjugate, which contains the cyclam based compound of the general formula (I) and/or the conjugate and/or the coordination compound according to the present invention, and a targeting vector, selected from the group comprising bis(phosphonates); groups capable of fluorescence, for example fluoresceins, rhodamines or the boron-dipyrromethene (BODIPY) type substances, preferably emitting in the red or near infra-red (NIR) part of the spectrum, most preferably fluorescein and rhodamine; oligopeptides of 1 to 15 aminoacids; antibodies or fragments thereof, especially the Fab antigen binding fragments; folic acid;

biotin; compounds targeting to PSMA receptor, preferably urea derivatives or organo-phosphorous compounds; cyclodextrins; dendrimers, preferably PAMAM dendrimers; hydrophilic polymers on the basis of derivatives of acrylic acid, especially hydroxoalkylamides of acrylic acid, preferably hydroxoalkylamides of acrylic acid  
 5 modified with amino groups and/or maleimido groups, most preferably the block copolymer of poly[N-(2-hydroxopropyl)methacrylamide and poly-L-lysine, poly[N-(2-hydroxopropyl)methacrylamide with free amino groups and poly[N-(2-hydroxopropyl)methacrylamide with free maleimide groups. Cyclodextrins, dendrimers and hydrophilic polymers based on derivatives of the acrylic acid are preferably modified  
 10 with functional groups allowing for further conjugations of targeting vectors, serving thus for, not limited to, to increase the number of molecules of the compound of the general formula (I) in the targeting conjugate. Targeting conjugates containing Fab fragments of antibodies have, compared to the whole antibodies, faster pharmacokinetics in the organism, therefore, they are pharmacokinetically more preferred comparing to  
 15 antibodies. Targeting conjugates containing groups capable of fluorescence are suitable especially for targeted image-guided surgery (IGS).

The subject of the present invention is also a method of preparation of cyclam based compounds of the general formula (I) according to the present invention, wherein the  
 20 intermediate product of the general formula (IV),



wherein R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup> and R<sup>14</sup> are in the following combinations:

Intermediate	R <sup>11</sup>	R <sup>12</sup>	R <sup>13</sup>	R <sup>14</sup>
A	H	H	H	OH
B	H	H	H	<i>p</i> -nitrobenzyl
C	H	H	H	-CH <sub>2</sub> N(benzyl) <sub>2</sub>
D	H	H	H	-(CH <sub>2</sub> ) <sub>2</sub> COOH
E	H	-(CH <sub>2</sub> ) <sub>4</sub> COOH	H	H
F	OH	-(CH <sub>2</sub> ) <sub>3</sub> NH(4-dibenzylcyclooctyn	OH	OH

		amidobutoxyl)		
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or a compound selected from the group containing methylene-bis(phosphinic acid), phosphorous acid, hydroxymethylphosphinic acid, reacts with a cyclam derivative and aldehyde in aqueous solution of an acid with the concentration within the range of from 5 10 to 40 % (weight), preferably from 18 to 36 % (weight), at the temperature in the range of from 40 °C to 80 °C for the period of time of at least 12 hours;

wherein the cyclam derivative is 1,4,8,11-tetraazacyclotetradecane and derivatives thereof and 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane and derivatives thereof, preferably the cyclam derivative is selected from the group containing 1,4,8,11- 10 tetraazacyclotetradecane, 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane, 1,4,8-trimethyl-1,4,8,11-tetraazacyclotetradecane, 1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane, 1,8-dibenzyl-1,4,8,11-tetraazacyclotetradecane, 1,4-dibenzyl-1,4,8,11-tetraazacyclotetradecane, 6,13-dimethyl-6,13-dinitro-1,4,8,11-tetraazacyclotetradecane; the aldehyde is selected from the group containing acetaldehyde, formaldehyde and para- 15 formaldehyde, and the acid is preferably HCl and/or CF<sub>3</sub>COOH; and wherein the reaction may be preceded by the step of preparation of the intermediate product.

In one embodiment of the method of preparation of the cyclam based compounds of the general formula (I) according to the present invention, the resulting product is further 20 subjected to a reduction reaction. Preferably, the reduction reaction is performed as a hydrogenation in the presence of catalysts, reduction with hydride complexes, reduction with metals in acid solutions or using sulfides.

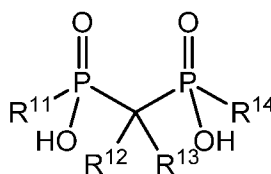
In another embodiment of the method of preparation of the cyclam based compounds of the general formula (I) according to the present invention, the resulting product is further 25 subjected to an oxidation reaction. Preferably, the oxidation reaction is performed with oxidation agents selected from the group containing Cl<sub>2</sub>, I<sub>2</sub> or Br<sub>2</sub> in water or in organic solvents, oxygen-containing oxidation agents, such as hydrogen peroxide or (organic) peroxyacids or salts thereof, metal ions in oxidation states capable of reduction, such as 30 Hg<sup>2+</sup> or Cu<sup>2+</sup>, or (organic) oxoacids of iodine in high formal oxidation degrees of +III or +V.

In another embodiment of method of preparation of cyclam based compounds of the general formula (I) according to the present invention, the resulting product is further subjected to reaction with an aldehyde; and eventually with an intermediate product of the general formula (IV), or with a compound, selected from the group containing  
 5 methylene-bis(phosphinic acid), phosphorous acid, alkylphosphinic acid or arylphosphinic acid, preferably hydroxymethylphosphinic acid; in aqueous solution of an acid, preferably HCl or CF<sub>3</sub>COOH.

In another embodiment of the method of preparation of cyclam based compounds of the general formula (I) according to the present invention, the reduction step is followed by a  
 10 step when the product of the reduction undergoes a reaction with CCl<sub>4</sub> or COCl<sub>2</sub>.

In another embodiment of the method of preparation of cyclam based compounds of the general formula (I) according to the present invention, the reduction step is followed by a  
 15 step when the resulting product of the reduction undergoes a reaction with NaN<sub>3</sub>.

The subject of the present invention is also an intermediate product of the general formula (IV),



(IV)

20

wherein R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup> and R<sup>14</sup> are in the following combinations:

Intermediate	R <sup>11</sup>	R <sup>12</sup>	R <sup>13</sup>	R <sup>14</sup>
B	H	H	H	<i>p</i> -nitrobenzyl
C	H	H	H	-CH <sub>2</sub> N(benzyl) <sub>2</sub>
D	H	H	H	-(CH <sub>2</sub> ) <sub>2</sub> COOH
E	H	-(CH <sub>2</sub> ) <sub>4</sub> COOH	H	H
F	OH	-(CH <sub>2</sub> ) <sub>3</sub> NH(4-dibenzylcyclooctynamidobutoxyl)	OH	OH

The subject of the present invention is also a pharmaceutical preparation, which contains at least one cyclam based compound according to the present invention and/or at least one conjugate according to the present invention and/or at least one coordination compound according to the present invention and/or at least one targeting conjugate according to the present invention, and a pharmaceutically acceptable substance. The dosage form of the pharmaceutical preparation is a form for administration by injection, most often as a bolus or as an infusion, preferably intravenously. Suitable pharmaceutically acceptable auxiliary substances are preferably selected from the group containing solvents (especially aqueous or saline solution), buffers (especially phosphate buffer, HEPES = 2-[4-(2-hydroxyethyl)piperazine-1-yl]ethanesulfonic acid), ionization additives, antioxidants, antimicrobial additives. A person skilled in the art would be able, without exerting inventive activity, to determine which adjuvans to choose.

Subject of the present invention is also the cyclam based compound according to the present invention and/or at least one conjugate according to the present invention and/or at least one coordination compound according to the present invention and/or at least one targeting conjugate according to the present invention and/or the pharmaceutical preparation according to the present invention, for use as a medicament, preferably in the treatment of tumor diseases, inflammations, and/or atherosclerosis. Especially coordination compounds with metal radioisotopes according to the present invention, preferably with copper, lutetium, bismuth and yttrium radioisotopes, are suitable for the use as medicaments in radiotherapy. Cyclam based compounds according to the present invention were successfully tested *in vivo* in rodents. It results from the tests performed that those substances are neither deposited nor decomposed anywhere in the body and they are excreted from the body in a normal manner (urine, faeces). Therefore, they are suitable for medical applications.

The subject of the present invention is also the cyclam based compound according to any of the claims 1 thru 5 and/or at least one conjugate according to the present invention and/or at least one coordination compound according to the present invention and/or at least one targeting conjugate according to the present invention and/or the pharmaceutical preparation according to the present invention, for use as a contrast agent in medical diagnostics, preferably in the diagnostics of tumor diseases, inflammations, and/or atherosclerosis. For luminescence methods in medical diagnostics, coordination

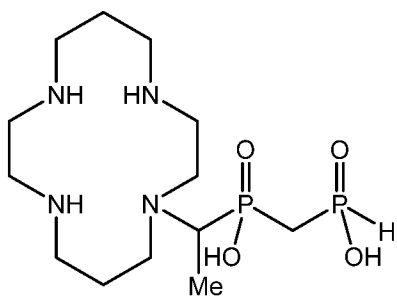
compounds according to the present invention containing cations of trivalent lanthanides are especially suitable; for magnetic nuclear resonance (NMR, MRI), coordination compounds of gadolinium are suitable.

- 5 The subject of the present invention is the use of cyclam based compounds according to the present invention and/or of the conjugate according to the present invention and/or of the coordination compound according to the present invention and/or of the targeting conjugate according to the present invention and/or of the pharmaceutical preparation according to the present invention, in radiochemistry as markers or precursors of markers  
10 when labelling with the use of radioisotopes of copper, lutetium, bismuth and yttrium, preferably using radioisotopes of copper, more preferably  $^{64}\text{Cu}$ .

The subject of the present invention is the use of cyclam based compounds according to the present invention and/or of the conjugate according to the present invention and/or of  
15 the coordination compound according to the present invention and/or of the targeting conjugate according to the present invention, for the complexation and/or purification of copper radioisotopes.

### Examples

- 20 Example 1: *Synthesis of compound 1*



- To a glass vial (4 mL), 1,4,8,11-tetraazacyclotetradecane (205 mg; 1.02 mmol; 5.0 equiv.) was added. Methylene-bis(phosphinic acid) (85.6 mg; 594  $\mu\text{mol}$ ; 2.9 equiv.), acetaldehyde (9.0 mg; 205  $\mu\text{mol}$ ; 1.0 equiv.) and aqueous HCl (6 M; 2 mL)  
25 were subsequently added and the resulting suspension was stirred at 80 °C overnight. The mixture was evaporated to dryness and several times co-evaporated with  $\text{H}_2\text{O}$ . Crude product was purified by ion exchange chromatography (DOWEX 50;  $\text{H}^+$ -form;  $\text{H}_2\text{O} \rightarrow 10\%$  aqueous pyridine). The pyridine fraction with product was evaporated to dryness and further co-evaporated with  $\text{H}_2\text{O}$  (25 mL). The residue was re-dissolved in

H<sub>2</sub>O (25 mL) and subsequently lyophilized. Product was obtained as white substance (35.0 mg; 40%; 1 step, based on acetaldehyde).

**NMR (D<sub>2</sub>O):** <sup>1</sup>H δ 1.16 (CH<sub>3</sub>, dd, 3H, <sup>3</sup>J<sub>HP</sub> = 15 Hz, <sup>2</sup>J<sub>HH</sub> = 7 Hz); 1.73–2.00 (P—CH<sub>2</sub>—P, CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 5H); 2.08 (P—CH<sub>2</sub>—P, dddd, 1H, <sup>2</sup>J<sub>HH</sub> = 19 Hz, <sup>2</sup>J<sub>HP</sub> = 16 Hz, <sup>2</sup>J<sub>HP</sub> = 12 Hz, <sup>3</sup>J<sub>HH</sub> = 4 Hz); 2.52–3.24 (CH<sub>2</sub>—N, m, 16H); 3.37 (CH—CH<sub>3</sub>, dq, 1H, <sup>2</sup>J<sub>HP</sub> = 14 Hz, <sup>2</sup>J<sub>HH</sub> = 7 Hz); 7.12 (PH, dd, <sup>1</sup>J<sub>HP</sub> = 534 Hz, <sup>3</sup>J<sub>HH</sub> = 4 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 6.3 (CH<sub>3</sub>, bm); 23.9 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 26.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 34.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 78 Hz, <sup>1</sup>J<sub>CP</sub> = 73 Hz); 44.8 (*cycle*, s); 46.4 (*cycle*, s); 46.5 (*cycle*, s); 46.7 (*cycle*, s); 49.4 (*cycle*, s); 49.6 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 10 Hz); 50.0 (*cycle*, s); 50.6 (*cycle*, s); 52.0 (N—CH—P, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); <sup>31</sup>P δ 19.1 (PH, dtd, 1P, <sup>1</sup>J<sub>PH</sub> = 534 Hz, <sup>2</sup>J<sub>PH</sub> = 16 Hz, <sup>2</sup>J<sub>PP</sub> = 4 Hz); 35.5 (N—CH—P, m, 1P).

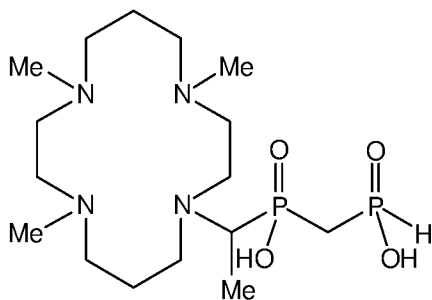
**MS:** (–) 368.8 [M–H<sup>+</sup>]<sup>–</sup>. (+) 371.0 [M+H<sup>+</sup>]<sup>+</sup>; 392.9 [M+Na<sup>+</sup>]<sup>+</sup>; 408.9 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*-PrOH–conc. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.7.

**EA** (C<sub>13</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>P<sub>2</sub>·3.5H<sub>2</sub>O, M<sub>R</sub> = 433.4): C 36.0 (35.6); H 9.1 (8.9); N 12.9 (13.2).

15

#### Example 2: Synthesis of compound 2



To a glass vial (4 mL), tetrahydrochloride of 1,4,8-trimethyl-1,4,8,11-tetraazaacyclotetradecane (65.2 mg; 168 μmol; 1.0 equiv.) was added. Methylenebis(phosphinic acid) (73.2 mg; 508 μmol; 3.0 equiv.), acetaldehyde (8.1 mg; 184 μmol; 1.1 equiv.) and aqueous HCl (6 M; 2 mL) were subsequently added and the resulting suspension was stirred at 60 °C for two days. The mixture was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. Crude product was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). The pyridine fraction with product was evaporated to dryness and further co-evaporated with H<sub>2</sub>O (25 mL). The residue was re-dissolved in H<sub>2</sub>O (55 mL) and subsequently lyophilized. Product was obtained as a white substance (48.7 mg; 63%; 1 step, based on acetaldehyde).

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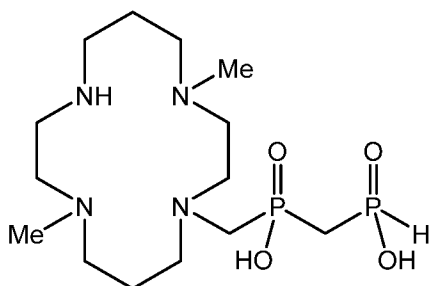
NMR (D<sub>2</sub>O): <sup>1</sup>H δ 1.19 (CH<sub>3</sub>—C, dd, 3H, <sup>3</sup>J<sub>HP</sub> = 16 Hz, <sup>2</sup>J<sub>HH</sub> = 7 Hz); 1.68 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 4H); 1.81 (P—CH<sub>2</sub>—P, m, 1H); 1.98 (P—CH<sub>2</sub>—P, m, 1H); 2.25 (CH<sub>3</sub>—N, m, 3H); 2.27 (CH<sub>3</sub>—N, s, 6H); 2.48–3.16 (CH<sub>2</sub>—N, m, 16H); 3.41 (CH—CH<sub>3</sub>, dq, 1H, <sup>2</sup>J<sub>HP</sub> = 15 Hz, <sup>2</sup>J<sub>HH</sub> = 7 Hz); 7.13 (PH, dm, <sup>1</sup>J<sub>HP</sub> = 533 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 7.1 (CH<sub>3</sub>—C, s); 21.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 22.7 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 35.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 77 Hz, <sup>1</sup>J<sub>CP</sub> = 73 Hz); 43.6 (CH<sub>3</sub>—N, s); 43.7 (CH<sub>3</sub>—N, s); 43.9 (CH<sub>3</sub>—N, s); 46.2 (cycle, s); 48.2 (cycle, s); 50.0 (cycle, s); 50.8 (cycle, d, <sup>3</sup>J<sub>CP</sub> = 8 Hz); 51.3 (cycle, d, <sup>3</sup>J<sub>CP</sub> = 9 Hz); 51.9 (cycle, s); 53.5 (cycle, s); 53.6 (cycle, s); 56.5 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); <sup>31</sup>P δ 19.7 (PH, dtd, 1P, <sup>1</sup>J<sub>PH</sub> = 533 Hz, <sup>2</sup>J<sub>PH</sub> = 16 Hz, <sup>2</sup>J<sub>PP</sub> = 3 Hz); 32.1 (N—CH—P, m, 1P).

MS: (–) 410.9 [M–H]<sup>–</sup>. (+) 413.1 [M+H]<sup>+</sup>; 435.1 [M+Na]<sup>+</sup>; 451.0 [M+K]<sup>+</sup>.

TLC (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 4:1): R<sub>f</sub> = 0.3.

EA (C<sub>16</sub>H<sub>38</sub>N<sub>4</sub>O<sub>4</sub>P<sub>2</sub>·2.5H<sub>2</sub>O, M<sub>R</sub> = 457.4): C 42.0 (42.3); H 9.5 (9.1); N 12.3 (12.2).

15 Example 3: *Synthesis of compound 3*



To a glass flask (25 ml), tetrahydrochloride of 1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane (1.56 mg; 4.17 mmol; 2.5 equiv.) was added. Methylenebis(phosphinic acid) (480 mg; 3.33 mmol; 2.0 equiv.), paraformaldehyde (50 mg; 1.67 mmol; 1.0 equiv.) and aqueous HCl (6 M; 5 mL) were subsequently added and the resulting suspension was stirred at 80 °C overnight. The mixture was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. Crude product was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). The pyridine fraction with product was evaporated to dryness and further co-evaporated several times with H<sub>2</sub>O. The crude product was further purified by column chromatography (SiO<sub>2</sub>; EtOH–conc. NH<sub>4</sub>OH 4:1; R<sub>f</sub> = 0.4). Fractions with product were combined and evaporated to dryness. The residue was suspended in H<sub>2</sub>O (25 mL), treated with small amounts of charcoal and filtered through syringe microfilter (Millipore; 0.22 μm). The filtrate was re-purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-

form; H<sub>2</sub>O → 10% aqueous pyridine). The pyridine fraction with product was evaporated to dryness and further co-evaporated several times with H<sub>2</sub>O. The residue was re-dissolved in H<sub>2</sub>O (25 mL) and subsequently lyophilized. Product was obtained as a white substance (215 mg; 29%; 1 step, based on paraformaldehyde).

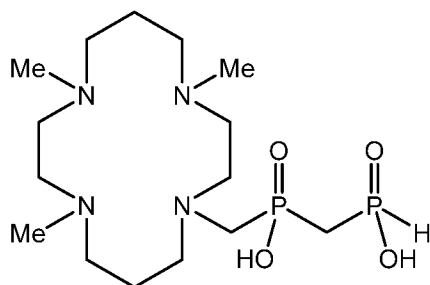
5 **NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.70 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 1.76 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.03 (P—CH<sub>2</sub>—P, ddd, 2H, <sup>2</sup>J<sub>HP</sub> = 18 Hz, <sup>2</sup>J<sub>HP</sub> = 16 Hz, <sup>3</sup>J<sub>HH</sub> = 2 Hz); 2.24 (CH<sub>3</sub>, s, 3H); 2.30 (CH<sub>3</sub>, s, 3H); 2.50 (*cycle*, t, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.57 (*cycle*, m, 4H); 2.66 (*cycle*, t, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.69–2.82 (*cycle*, N—CH<sub>2</sub>—P, m, 10H); 7.14 (PH, dt, <sup>1</sup>J<sub>HP</sub> = 531 Hz, <sup>3</sup>J<sub>HH</sub> = 2 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 23.0 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 23.6 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 35.9 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 77 Hz, <sup>1</sup>J<sub>CP</sub> = 75 Hz); 43.5 (*cycle*, s); 43.2 (CH<sub>3</sub>, s); 43.8 (CH<sub>3</sub>, s); 44.8 (*cycle*, s); 51.1 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 8 Hz); 52.2 (*cycle*, s); 53.1 (*cycle*, s); 53.4 (*cycle*, s); 53.5 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 6 Hz); 53.7 (*cycle*, s); 56.1 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); <sup>31</sup>P δ 19.8 (PH, dtd, 1P, <sup>1</sup>J<sub>PH</sub> = 531 Hz, <sup>2</sup>J<sub>PH</sub> = 18 Hz, <sup>2</sup>J<sub>PP</sub> = 3 Hz); 32.5 (N—CH—P, m, 1P).

10 **MS**: (–) 382.6 [M–H<sup>+</sup>]<sup>–</sup>. (+) 384.6 [M+H<sup>+</sup>]<sup>+</sup>; 406.8 [M+Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 4:1): R<sub>f</sub> = 0.4.

**EA** (C<sub>14</sub>H<sub>34</sub>N<sub>4</sub>O<sub>4</sub>P<sub>2</sub>·3.5H<sub>2</sub>O, M<sub>R</sub> = 447.5): C 37.6 (37.5); H 9.2 (9.0); N 12.5 (12.4).

#### Example 4: Synthesis of compound 4



20 To a glass vial (4 mL), tetrahydrochloride of 1,4,8-trimethyl-1,4,8,11-tetraazaacyclotetradecane (37.8 mg; 99.7 μmol; 1.0 equiv.) was added. Methylenebis(phosphonic acid) (55.6 mg; 386 μmol; 3.9 equiv.), paraformaldehyde (5.6 mg; 210 μmol; 2.1 equiv.) and aqueous HCl (6 M; 2 mL) were subsequently added and the

25 resulting suspension was stirred at 60 °C for two days. The mixture was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The crude product was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). The pyridine fraction with product was evaporated to dryness and further co-evaporated with H<sub>2</sub>O (25 mL). The residue was re-dissolved in H<sub>2</sub>O (25 mL) and subsequently

lyophilized. Product was obtained as a white substance (35.4 mg; 82%; 1 step, based on paraformaldehyde).

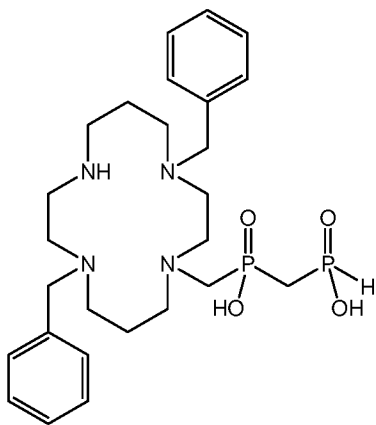
**NMR** ( $D_2O$ ):  $^1H$   $\delta$  1.71 ( $CH_2-CH_2-CH_2$ , m, 4H); 2.04 (P- $CH_2$ -P, ddd, 2H,  $^2J_{HP} = 18$  Hz,  $^2J_{HP} = 16$  Hz,  $^3J_{HH} = 2$  Hz); 2.24 ( $CH_3$ , m, 6H); 2.27 ( $CH_3$ , s, 3H); 2.47–2.64 (cycle, m, 12H); 2.75 (cycle, m, 2H); 2.81 (N- $CH_2$ -P, d, 2H,  $^3J_{HH} = 9$  Hz); 2.84 (cycle, m, 2H); 7.14 (PH, dt,  $^1J_{HP} = 531$  Hz,  $^3J_{HH} = 2$  Hz);  $^{13}C\{^1H\}$   $\delta$  20.2 ( $CH_2-CH_2-CH_2$ , s); 20.6 ( $CH_2-CH_2-CH_2$ , s); 35.4 (P- $CH_2$ -P, t,  $^1J_{CP} = 77$  Hz); 43.5 ( $CH_3$ , s); 43.7 ( $CH_3$ , s); 43.8 ( $CH_3$ , s); 49.0 (cycle, s); 49.2 (cycle, s); 49.6 (cycle, s); 50.0 (cycle, d,  $^3J_{CP} = 8$  Hz); 52.1 (cycle, d,  $^3J_{CP} = 8$  Hz); 52.2 (cycle, s); 53.4 (cycle, s); 53.5 (cycle, s); 56.7 (N- $CH_2$ -P, d,  $^1J_{CP} = 109$  Hz);  $^{31}P$   $\delta$  19.7 (PH, dtd, 1P,  $^1J_{PH} = 531$  Hz,  $^2J_{PH} = 18$  Hz,  $^2J_{PP} = 3$  Hz); 32.1 (N-CH-P, m, 1P).

**MS:** (-) 396.8  $[M-H]^+$ ; (+) 399.0  $[M+H]^+$ ; 421.0  $[M+Na]^+$ ; 437.0  $[M+K]^+$ .

**TLC** ( $SiO_2$ , EtOH-conc.  $NH_4OH$  4:1):  $R_f = 0.2$ .

**EA** ( $C_{14}H_{34}N_4O_4P_2 \cdot 2H_2O$ ,  $M_R = 434.5$ ): C 41.5 (41.8); H 9.3 (9.1); N 12.9 (12.9).

#### Example 5: Synthesis of compound 5



To a glass vial (20 mL), tetrahydrochloride of 1,8-dibenzyl-1,4,8,11-tetraazacyclotetradecane (506 mg; 959  $\mu$ mol; 2.0 equiv.) was added. Methylenebis(phosphinic acid) (136 mg; 944  $\mu$ mol; 2.0 equiv.),  $CF_3COOH$  (5 mL), aqueous HCl (12 M; 5 mL) and paraformaldehyde (20.9 mg; 474  $\mu$ mol; 1.0 equiv.) were subsequently added and the resulting suspension was stirred at 80 °C for two days. The mixture was evaporated to dryness and several times co-evaporated with  $H_2O$ . The crude product was further purified by column chromatography ( $SiO_2$ ; EtOH-conc. aq.  $NH_4OH$  5:1;  $R_f = 0.6$ ).

The fractions with product were combined and evaporated to dryness. The residue was suspended in  $H_2O$  (25 mL), treated with small amounts of charcoal and filtered through

syringe microfilter (Millipore; 0.22  $\mu\text{m}$ ). The filtrate was re-purified by ion exchange chromatography (DOWEX 50;  $\text{H}^+$ -form;  $\text{H}_2\text{O} \rightarrow 10\%$  aqueous pyridine). The pyridine fraction with product was evaporated to dryness and further co-evaporated several times with  $\text{H}_2\text{O}$ . The residue was re-dissolved in  $\text{H}_2\text{O}$  (100 mL) and subsequently lyophilized.

5 Product was obtained as a white substance (146 mg; 54%; 1 step, based on paraformaldehyde).

NMR ( $\text{D}_2\text{O}$ ):  $\delta$  1.82 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , m, 4H); 2.05 (P— $\text{CH}_2$ —P, dd, 2H,  $^2J_{\text{HP}} = 18$  Hz,  $^2J_{\text{HP}} = 15$  Hz); 2.46–2.61 (*cycle*, m, 6H); 2.74–3.12 (*cycle*, N— $\text{CH}_2$ —P, m, 10H); 3.68 ( $\text{CH}_2$ —Ph, s, 2H); 3.75 ( $\text{CH}_2$ —Ph, s, 2H); 7.14 (PH, d,  $^1J_{\text{HP}} = 529$  Hz); 7.22–7.35 (Ph, m, 10H);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  24.1 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , s); 27.2 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , s); 37.1 (P— $\text{CH}_2$ —P, dd,  $^1J_{\text{CP}} = 79$  Hz,  $^1J_{\text{CP}} = 71$  Hz); 41.6 (*cycle*, s); 42.2 (*cycle*, s); 50.1 (*cycle*, d,  $^3J_{\text{CP}} = 7$  Hz); 52.2 (*cycle*, s); 53.3 (*cycle*, s); 53.4 (*cycle*, s); 53.8 (*cycle*, d,  $^3J_{\text{CP}} = 9$  Hz); 54.2 (*cycle*, s); 56.2 ( $\text{CH}_2$ —Ph, s); 58.8 (N— $\text{CH}_2$ —P, d,  $^1J_{\text{CP}} = 105$  Hz); 59.1 ( $\text{CH}_2$ —Ph, s); 124.1 (Ph, s); 126.4 (Ph, s); 128.1 (Ph, s); 128.3 (Ph, s); 128.9 (Ph, s); 130.8 (Ph, s); 138.1 (Ph, s); 139.1 (Ph, s);  $^{31}\text{P}$   $\delta$  26.4 (PH, dm, 1P,  $^1J_{\text{PH}} = 529$  Hz); 34.3 (N—CH—P, m, 1P).

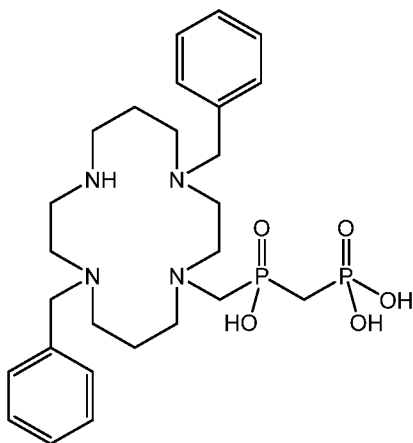
MS: (–) 535.1 [ $\text{M—H}^+$ ] $^-$ . (+) 537.1 [ $\text{M+H}^+$ ] $^+$ ; 559.2 [ $\text{M+Na}^+$ ] $^+$ ; 581.4 [ $\text{M—H}^++2\text{Na}^+$ ] $^+$ .

TLC ( $\text{SiO}_2$ , EtOH–conc.  $\text{NH}_4\text{OH}$  5:1):  $R_f = 0.6$ .

EA ( $\text{C}_{26}\text{H}_{42}\text{N}_4\text{O}_4\text{P}_2 \cdot 2\text{H}_2\text{O}$ ,  $M_R = 572.6$ ): C 54.5 (54.7); H 8.1 (7.7); N 9.8 (10.0).

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#### Example 6: Synthesis of compound 6



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In a glass flask (50 mL),  $5 \cdot 2\text{H}_2\text{O}$  (103 mg; 180  $\mu\text{mol}$ ; 1.0 equiv.) was dissolved in aqueous HCl (1%; 10 mL). Solution of  $\text{HgCl}_2$  (83.1 mg; 306  $\mu\text{mol}$ ; 1.7 equiv.) in  $\text{H}_2\text{O}$  (10 mL) was added and the mixture was stirred at 60  $^\circ\text{C}$  overnight. Precipitate was filtered off and the mother liquor was saturated with  $\text{H}_2\text{S}$ . Precipitate was

filtered off, washed with H<sub>2</sub>O and the filtrate was evaporated to dryness. The residue was further dried on vacuum pump and subsequently in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. Product was obtained as a white substance (106 mg; 84%; 1 step, based on 5·2H<sub>2</sub>O).

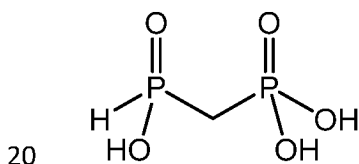
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.76 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 4 Hz); 1.88 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.03 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 18 Hz, <sup>2</sup>J<sub>HP</sub> = 14 Hz); 2.44 (*cycle*, m, 2H); 2.51–2.63 (*cycle*, m, 4H); 2.69–3.30 (*cycle*, N—CH<sub>2</sub>—P, m, 10H); 3.72 (CH<sub>2</sub>—Ph, s, 2H); 3.74 (CH<sub>2</sub>—Ph, s, 2H); 7.16 (*Ph*, m, 2H); 7.25–7.34 (*Ph*, m, 8H); <sup>13</sup>C{<sup>1</sup>H} δ 24.9 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 26.9 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 36.6 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 114 Hz, <sup>1</sup>J<sub>CP</sub> = 70 Hz); 40.8 (*cycle*, s); 42.2 (*cycle*, s); 50.3 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 7 Hz); 52.8 (*cycle*, s); 53.5 (*cycle*, s); 53.6 (*cycle*, s); 53.8 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 9 Hz); 54.8 (*cycle*, s); 55.3 (CH<sub>2</sub>—Ph, s); 58.8 (CH<sub>2</sub>—Ph, s); 60.1 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 106 Hz); 125.2 (*Ph*, s); 125.9 (*Ph*, s); 128.1 (*Ph*, s); 128.3 (*Ph*, s); 130.0 (*Ph*, s); 130.2 (*Ph*, s); 138.5 (*Ph*, s); 140.3 (*Ph*, s); <sup>31</sup>P{<sup>1</sup>H} δ 21.4 (HO—P—OH, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 33.3 (N—CH—P, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz).

**MS:** (–) 551.1 [M–H<sup>+</sup>]<sup>–</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 5:1): R<sub>f</sub> = 0.2.

**EA** (C<sub>26</sub>H<sub>42</sub>N<sub>4</sub>O<sub>5</sub>P<sub>2</sub>·3.5HCl·H<sub>2</sub>O, M<sub>R</sub> = 698.2): C 44.7 (45.0); H 6.9 (6.7); N 8.0 (8.1).

#### Example 7: Synthesis of intermediate product A



In a glass flask, methylene-bis(phosphinic acid) (2.50 g; 17.4 mmol; 1.0 equiv.) was dissolved in H<sub>2</sub>O (50 mL). Solution of HgCl<sub>2</sub> (5.90 g; 21.7 mmol; 1.2 equiv.) in H<sub>2</sub>O (50 mL) was added and the mixture was stirred at 60 °C for three days. Precipitate was filtered off, washed with H<sub>2</sub>O and the mother liquor was saturated with H<sub>2</sub>S. Precipitate was filtered off, washed with H<sub>2</sub>O and the filtrate was evaporated to dryness. The crude product was further purified by column chromatography (SiO<sub>2</sub>; *i*PrOH–conc. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3; R<sub>f</sub> = 0.2). The fractions with product were combined and evaporated to dryness. The residue was suspended in H<sub>2</sub>O (25 mL), treated with small amounts of charcoal and filtered through syringe microfilter (Millipore; 0.22 μm). The filtrate was re-purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Aqueous fraction with product was evaporated to

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dryness and the residue was further dried on vacuum pump and subsequently in vacuum desiccator over  $P_2O_5$ . Product was obtained as a white substance (1.24 g; 42%; 1 step, based on methylene-bis(phosphinic acid)).

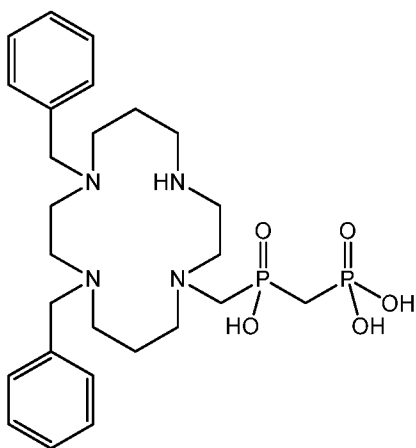
**NMR** ( $D_2O$ ):  $^1H$   $\delta$  1.93 ( $CH_2-P$ , tm, 2H,  $^2J_{HP} = 18$  Hz); 7.10 (PH, dm, 1H,  $^1J_{HP} = 526$  Hz);  $^{13}C\{^1H\}$   $\delta$  34.6 ( $CH_2$ , dd,  $^1J_{CP} = 114$  Hz,  $^1J_{CP} = 78$  Hz);  $^{31}P$   $\delta$  12.3 (HO—P—OH, td,  $^2J_{PH} = 18$  Hz,  $^2J_{PP} = 4$  Hz); 24.9 (HO—P—H, dtd,  $^1J_{PH} = 526$  Hz,  $^2J_{PH} = 18$  Hz,  $^2J_{PP} = 4$  Hz).

**MS**: (–) 158.6  $[M-H^+]^-$ . (+) 160.8  $[M+H^+]^+$ .

**TLC** ( $SiO_2$ , EtOH–conc.  $NH_4OH$  1:1):  $R_f = 0.2$ .

**EA** ( $CH_6O_5P_2 \cdot 0.5H_2O$ ,  $M_R = 169.0$ ): C 7.1 (7.1); H 4.2 (4.0).

Example 8: *Synthesis of compound 7*



To a glass vial (20 ml), tetrahydrochloride of 1,4-dibenzyl-1,4,8,11-tetraazacyclotetradecane (554 mg; 1.05  $\mu$ mol; 2.0 equiv.) was added. The intermediate  $A \cdot 0.5H_2O$  (355 mg; 1.05 mmol; 2.0 equiv.),  $CF_3COOH$  (5 mL), aqueous HCl (12 M; 5 mL) and paraformaldehyde (23.1 mg; 525  $\mu$ mol; 1.0 equiv.) were subsequently added and the resulting suspension was stirred at 80 °C for two days. The mixture was evaporated to dryness and several times co-evaporated with  $H_2O$ . The crude product was further purified by column chromatography ( $SiO_2$ ; EtOH–conc.  $NH_4OH$  5:1;  $R_f = 0.3$ ). The fractions with product were combined and evaporated to dryness. The residue was suspended in  $H_2O$  (25 mL), treated with small amounts of charcoal and filtered through syringe microfilter (Millipore; 0.22  $\mu$ m). The filtrate was re-purified by ion exchange chromatography (DOWEX 50;  $H^+$ -form;  $H_2O \rightarrow 10\%$  aqueous pyridine). The pyridine fraction with product was evaporated to dryness and further co-evaporated several times with  $H_2O$ . The residue was re-dissolved in  $H_2O$  (100 mL) and subsequently lyophilized.

Product was obtained as a white substance (198 mg; 62%; 1 step, based on paraformaldehyde).

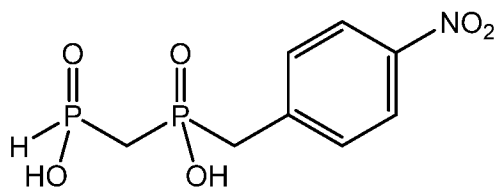
**NMR** ( $D_2O$ ):  $^1H$   $\delta$  1.74–1.92 ( $CH_2-CH_2-CH_2$ , bm, 4H); 2.09 (P– $CH_2$ –P, t, 2H,  $^2J_{HP} = 16$  Hz); 2.44–2.49 *cycle*, bm, 3H); 2.54 (*cycle*, m, 1H); 2.64–2.67 (*cycle*, m, 3H); 2.82–3.16 (*cycle*, N– $CH_2$ –P, bm, 11H); 3.84 ( $CH_2$ –Ph, s, 2H); 3.96 ( $CH_2$ –Ph, s, 2H); 7.14–7.33 (Ph, bm, 6H); 7.37 (Ph, m, 2H); 7.44 (Ph, m, 2H);  $^{13}C\{^1H\}$   $\delta$  25.5 ( $CH_2-CH_2-CH_2$ , s); 25.9 ( $CH_2-CH_2-CH_2$ , s); 36.6 (P– $CH_2$ –P, dd,  $^1J_{CP} = 118$  Hz,  $^1J_{CP} = 83$  Hz); 41.3 (*cycle*, s); 41.4 (*cycle*, s); 48.9 (*cycle*, s); 49.7 (*cycle*, d,  $^3J_{CP} = 10$  Hz); 52.4 (*cycle*, s); 54.6 (*cycle*, s); 55.5 (*cycle*, d,  $^3J_{CP} = 10$  Hz); 55.8 (*cycle*, s); 59.8 ( $CH_2$ –Ph, s); 60.1 (N– $CH_2$ –P, d,  $^1J_{CP} = 109$  Hz); 61.3 ( $CH_2$ –Ph, s); 126.0 (*Ph*, s); 127.4 (*Ph*, s); 130.2 (*Ph*, s); 130.7 (*Ph*, s); 131.4 (*Ph*, s); 132.2 (*Ph*, s); 134.2 (*Ph*, s); 137.6 (*Ph*, s);  $^{31}P\{^1H\}$   $\delta$  19.2 (HO–P–OH, d, 1P,  $^2J_{PP} = 7$  Hz); 36.2 (N–CH–P, d, 1P,  $^2J_{PP} = 7$  Hz).

**MS:** (–) 535.4  $[M-H^+]^-$ . (+) 537.3  $[M+H^+]^+$ ; 559.5  $[M+Na^+]^+$ ; 581.2  $[M-H^++2Na^+]^+$ .

**TLC** ( $SiO_2$ , EtOH–conc.  $NH_4OH$  5:1):  $R_f = 0.3$ .

**EA** ( $C_{26}H_{42}N_4O_4P_2 \cdot 4H_2O$ ,  $M_R = 608.6$ ): C 50.9 (51.3); H 8.0 (8.3); N 9.4 (9.2).

Example 9: *Synthesis of intermediate product B*



In a glass flask (100 mL), methylene-bis(phosphinic acid) (1.04 g; 7.22 mol; 1.0 equiv.) was suspended in dry  $CH_2Cl_2$  (25 mL). *N,N*-diisopropylethylamine (9.90 mL; 57.8 mmol; 8.0 equiv.) was added and the flask was washed by gentle stream of argon gas. Then trimethylsilylchloride was added (5.50 mL; 43.5 mmol; 6.0 equiv.) and the resulting mixture was stirred at room temperature for one hour. Solution of 4-nitrobenzylbromide (1.87 g; 8.66 mmol; 1.2 equiv.) in dry  $CH_2Cl_2$  (20 mL) was added and the resulting mixture was stirred at room temperature for three days. Reaction was stopped by addition of EtOH (25 mL). The mixture was evaporated to dryness and further co-evaporated several times with EtOH. The residue was suspended in  $H_2O$  (100 mL). The solid material was filtered off and the filtrate was evaporated to dryness. Crude product was purified by ion exchange chromatography (DOWEX 1;  $OH^-$ -form;  $H_2O \rightarrow 50\% AcOH \rightarrow 3\% HCl$ ). The latter fraction was evaporated to

dryness and further purified by ion exchange chromatography (Amberlite CG50; H<sup>+</sup>-form; H<sub>2</sub>O). Fraction with pure product were combined and evaporated to dryness and the residue was further dried on vacuum pump and subsequently in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. Product was obtained as a yellow substance (1.40 g; 69%; 1 step, based on

5 methylene-bis(phosphinic acid).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 2.28 (P—CH<sub>2</sub>—P, m, 2H); 3.29 (CH<sub>2</sub>—C, d, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz); 7.16 (PH, d, 1H, <sup>1</sup>J<sub>HP</sub> = 532 Hz); 7.53 (CH—C—CH<sub>2</sub>, d, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz); 8.21 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 35.3 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 78 Hz); 40.4 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 86 Hz); 124.3 (CH—C—N, s); 131.3 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 4 Hz); 144.0 (CH—C—CH<sub>2</sub>, d, <sup>2</sup>J<sub>CP</sub> = 8 Hz); 146.7 (CH—C—N, s); <sup>31</sup>P δ 18.7 (PH, dt, 1P, <sup>1</sup>J<sub>PH</sub> = 532 Hz; <sup>2</sup>J<sub>PH</sub> = 18 Hz); 30.5 (CH<sub>2</sub>—P—CH<sub>2</sub>, p, 1P, <sup>2</sup>J<sub>PH</sub> = 17 Hz).

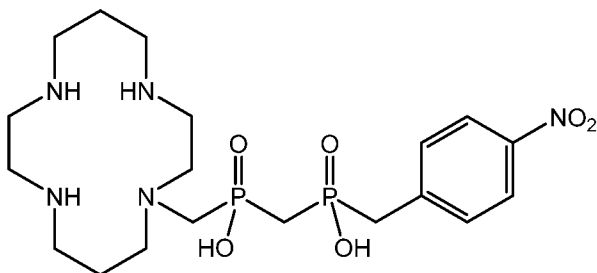
**MS:** (–) 277.5 [M–H<sup>+</sup>]<sup>–</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 5:1): R<sub>f</sub> = 0.5.

**EA** (C<sub>8</sub>H<sub>11</sub>NO<sub>6</sub>P<sub>2</sub>, M<sub>R</sub> = 279.1): C 34.4 (34.2); H 4.0 (3.9); N 5.0 (4.8).

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Example 10: *Synthesis of compound 8*



To a glass flask (100 ml), 1,4,8,11-tetraazacyclotetradecane (3.38 g; 16.9 mmol; 3.6 equiv.) was added. Intermediate **B** (1.31 g; 4.69 mmol; 1.0 equiv.), paraformaldehyde (169 mg; 5.63 mmol; 1.2 equiv.) and aqueous HCl (6 M; 60 mL) were subsequently added and the resulting mixture was stirred at 80 °C overnight. The mixture was evaporated to dryness and co-evaporated several times with H<sub>2</sub>O. The crude product was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). Pyridine fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 1; OH<sup>–</sup>-form; H<sub>2</sub>O → 10% aqueous AcOH). Acetate fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was further dried on vacuum pump and

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subsequently in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. Product was obtained as a white substance (1.67 g; 71%; 1 step, based on intermediate **B**).

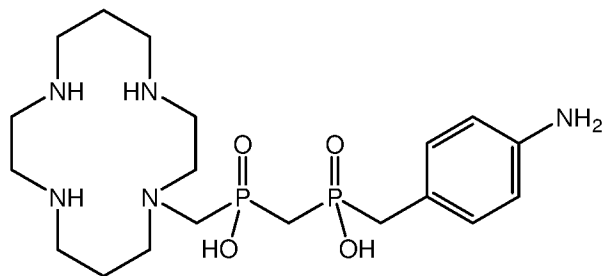
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.70 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 5 Hz); 1.76 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 5 Hz); 2.01 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.55–2.83 (cycle, N—CH<sub>2</sub>—P, m, 18H); 3.32 (CH<sub>2</sub>—C—CH, d, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz); 7.56 (CH—C—CH<sub>2</sub>, d, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz); 8.21 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 25.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 27.0 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.2 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 79 Hz); 40.8 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 86 Hz); 46.0 (cycle, s); 46.2 (cycle, s); 46.7 (cycle, s); 46.8 (cycle, s); 47.9 (cycle, s); 48.9 (cycle, s); 54.8 (cycle, s); 54.5 (cycle, s); 55.2 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 110 Hz); 124.3 (CH—C—N, s); 131.3 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 4 Hz); 144.6 (CH—C—CH<sub>2</sub>, s); 146.6 (CH—C—N, s); <sup>31</sup>P{<sup>1</sup>H} δ 31.6 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 32.9 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz).

**MS**: (–) 489.7 [M–H<sup>+</sup>]<sup>–</sup>. (+) 491.9 [M+H<sup>+</sup>]<sup>+</sup>; 513.9 [M+Na<sup>+</sup>]<sup>+</sup>; 529.8 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.7.

**EA** (C<sub>19</sub>H<sub>35</sub>N<sub>5</sub>O<sub>6</sub>P<sub>2</sub>·0.5H<sub>2</sub>O, M<sub>R</sub> = 500.5): C 45.6 (45.2); H 7.3 (7.6); N 14.0 (13.8).

Example 11: *Synthesis of compound 9*



In a glass flask (100 ml), **8**·0.5H<sub>2</sub>O (512 mg; 1.02 mmol; 1.0 equiv.) was dissolved in H<sub>2</sub>O and the resulting mixture was briefly washed with gaseous argon. Ammonium formate (1.98 g; 31.4 mmol; 31 equiv.) and Pd@C catalyst (10%; 54 mg) were subsequently added. Resulting suspension was stirred at 60 °C for three hours. Another portion of ammonium formate (658 mg; 10.4 mmol; 10 equiv.) and Pd@C catalyst (10%; 52 mg) was added and the mixture was further stirred at 60 °C for two days. The mixture was then evaporated to dryness and several times evaporated with H<sub>2</sub>O. The residue was dissolved in H<sub>2</sub>O, catalyst was filtered off and the filtrate was purified by ion exchange chromatography (DOWEX 1; OH<sup>–</sup>-forma; H<sub>2</sub>O → 10% aqueous AcOH). Acetate fraction with product was evaporated to dryness and then several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange

chromatography (DOWEX 50; H<sup>+</sup>-forma; H<sub>2</sub>O → 10% aqueous pyridine). Pyridine fraction with product was evaporated to dryness and further co-evaporated several times with H<sub>2</sub>O. The residue was re-dissolved in H<sub>2</sub>O (150 ml) and lyophilized. Product was obtained as an off-white substance (443 mg; 84%; 1 step; based on 8·0.5H<sub>2</sub>O).

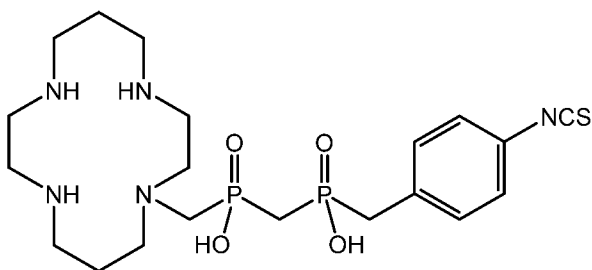
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.72 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 5 Hz); 1.79 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 5 Hz); 1.96 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.62–2.88 (*cycle*, N—CH<sub>2</sub>—P, m, 18H); 3.02 (CH<sub>2</sub>—C—CH, d, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz); 6.82 (CH—C—CH<sub>2</sub>, d, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); 7.18 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); <sup>13</sup>C{<sup>1</sup>H} 25.0 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 26.7 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 32.7 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 78 Hz); 39.4 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 91 Hz); 45.7 (*cycle*, s); 46.2 (*cycle*, s); 46.7 (*cycle*, s); 46.8 (*cycle*, s); 48.7 (*cycle*, s); 49.4 (*cycle*, s); 54.8 (*cycle*, s); 54.9 (*cycle*, s); 55.0 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); 117.3 (CH—C—N, s); 126.4 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 8 Hz); 131.4 (CH—C—CH<sub>2</sub>, s); 144.8 (CH—C—N, s); <sup>31</sup>P{<sup>1</sup>H} δ 33.7 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 34.6 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz).

**MS:** (–) 459.8 [M–H<sup>+</sup>]<sup>–</sup>. (+) 461.9 [M+H<sup>+</sup>]<sup>+</sup>; 483.9 [M+Na<sup>+</sup>]<sup>+</sup>; 499.9 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.6.

**EA** (C<sub>19</sub>H<sub>37</sub>N<sub>5</sub>O<sub>4</sub>P<sub>2</sub>·3H<sub>2</sub>O, M<sub>R</sub> = 515.5): C 44.3 (44.4); H 8.4 (8.6); N 13.6 (13.6).

20 Example 12: *Synthesis of compound 10*



In a glass vial (20 mL), **9**·3H<sub>2</sub>O (53.4 mg; 104 μmol; 1.0 equiv.) was dissolved in H<sub>2</sub>O. The solution was acidified by aqueous HCl (1.12 M; 185 μL; 207 μmol; 2.0 equiv.). Freshly prepared solution of CSCI<sub>2</sub> (16 μL; 209 μmol; 2.0 equiv.) in CCl<sub>4</sub> (5 mL) was then added and the resulting biphasic mixture was vigorously stirred at room temperature overnight. Organic layer was separated and the aqueous layer was extracted with CCl<sub>4</sub> (2×20 mL) and Et<sub>2</sub>O (1×20 mL). Aqueous layer was then separated and evaporated to dryness. The crude product was purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN). Fraction with product was

directly lyophilized. Product was obtained as a white substance (70.9 mg; 79%; 1 step; based on  $9 \cdot 3\text{H}_2\text{O}$ ).

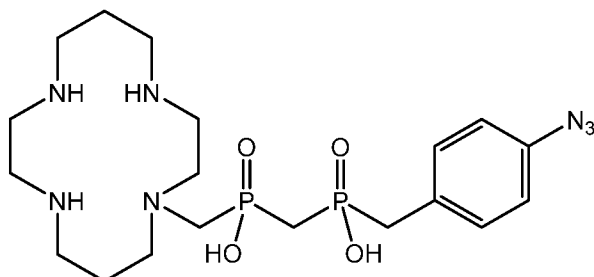
**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  2.19 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , m, 4H); 2.40 ( $\text{P—CH}_2\text{—P}$ , t, 2H,  $^2J_{\text{HP}} = 16$  Hz); 3.13–3.60 (*cycle*,  $\text{N—CH}_2\text{—P}$ ,  $\text{P—CH}_2\text{—C}$ , m, 20H); 7.36 ( $\text{CH}$ , m, 4H);  
 5  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  21.1 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , s); 21.7 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , s); 32.8 ( $\text{P—CH}_2\text{—P}$ , t,  $^1J_{\text{CP}} = 81$  Hz); 38.8 ( $\text{P—CH}_2\text{—C}$ , d,  $^1J_{\text{CP}} = 91$  Hz); 40.5 (*cycle*, s); 41.4 (*cycle*, s); 42.8 (*cycle*, s); 43.1 (*cycle*, s); 43.9 (*cycle*, s); 44.3 (*cycle*, s); 52.9 (*cycle*, s); 54.3 ( $\text{P—CH}_2\text{—N}$ , d,  $^1J_{\text{CP}} = 100$  Hz); 54.4 (*cycle*, s); 126.7 ( $\text{CH—C—N}$ , s); 130.1 ( $\text{CH—C—N}$ , s); 131.6 ( $\text{CH—C—CH}_2$ , d,  $^3J_{\text{CP}} = 5$  Hz); 132.9 ( $\text{CH—C—CH}_2$ , d,  $^2J_{\text{CP}} = 8$  Hz); 134.8 ( $\text{NCS}$ , s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  27.0 ( $\text{CH}_2\text{—P—CH}_2$ , bm, 1P); 39.1 ( $\text{CH}_2\text{—P—CH}_2$ , d, 1P,  $^2J_{\text{PP}} = 7$  Hz).

**MS**: (–) 501.8  $[\text{M—H}^+]^-$ . (+) 504.0  $[\text{M+H}^+]^+$ .

**EA** ( $\text{C}_{20}\text{H}_{35}\text{N}_5\text{O}_4\text{P}_2\text{S} \cdot \text{TFA} \cdot 3.5\text{H}_2\text{O}$ ,  $M_{\text{R}} = 863.3$ ): C 30.6 (30.8); H 5.0 (4.8); N 8.1 (8.0).

15

Example 13: *Synthesis of compound II*



In a glass vial (4 mL),  $9 \cdot 3\text{H}_2\text{O}$  (40.2 mg; 78.0  $\mu\text{mol}$ ; 1.3 equiv.) was dissolved in aqueous HCl (243.8 mM; 962  $\mu\text{L}$ ; 235  $\mu\text{mol}$ ; 4.0 equiv.) and the resulting mixture  
 20 was cooled in an ice bath (1 °C). Freshly prepared aqueous solution of  $\text{NaNO}_2$  was then gradually added (290 mM; 200  $\mu\text{L}$ ; 58.0  $\mu\text{mol}$ ; 1.0 equiv.; 20  $\mu\text{L}$  each three minutes). Freshly prepared aqueous solution of  $\text{NaN}_3$  (580 mM; 200  $\mu\text{L}$ ; 116  $\mu\text{mol}$ ; 2.0 equiv.) was then added. The reaction mixture was then stirred at room temperature for three hours. The crude product was purified by preparative HPLC (C8; gradient elution  
 25  $\text{H}_2\text{O}$ –0,1% TFA–MeCN). Fraction with product was directly lyophilized. Product was obtained as a white substance (29.5 mg; 57%; 1 step; based on  $9 \cdot 3\text{H}_2\text{O}$ ).

**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  2.07 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , p, 2H,  $^3J_{\text{HH}} = 6$  Hz); 2.13 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , p, 2H,  $^3J_{\text{HH}} = 7$  Hz); 2.20 ( $\text{P—CH}_2\text{—P}$ , t, 2H,  $^2J_{\text{HP}} = 16$  Hz); 2.80–3.57 (*cycle*,  $\text{N—CH}_2\text{—P}$ ,  $\text{P—CH}_2\text{—C}$ , bm, 20H); 7.11 ( $\text{CH—C—N}$ , d, 2H,  $^3J_{\text{HH}} = 8$  Hz); 7.34

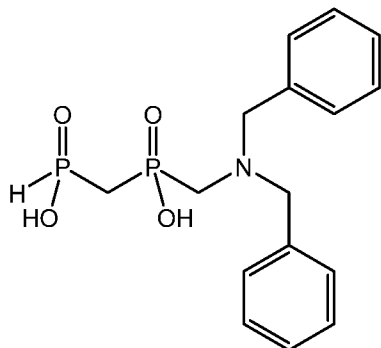
(CH—C—CH<sub>2</sub>, d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 22.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 22.7 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 32.3 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 80 Hz); 39.3 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 91 Hz); 42.4 (*cycle*, s); 43.5 (*cycle*, s); 44.3 (*cycle*, s); 45.1 (*cycle*, s); 45.4 (*cycle*, s); 46.0 (*cycle*, s); 53.7 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 105 Hz); 54.6 (*cycle*, s); 55.6 (*cycle*, s);  
 5 119.9 (CH—C—N, s); 130.9 (CH—C—CH<sub>2</sub>, d, <sup>2</sup>J<sub>CP</sub> = 8 Hz); 131.8 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz); 139.0 (CH—C—N, s); <sup>31</sup>P{<sup>1</sup>H} δ 29.6 (CH<sub>2</sub>—P—CH<sub>2</sub>, bm, 1P); 37.1 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz).

MS: (-) 485.8 [M—H<sup>+</sup>]<sup>-</sup>. (+) 488.0 [M+H<sup>+</sup>]<sup>+</sup>; 509.9 [M+Na<sup>+</sup>]<sup>+</sup>; 531.9 [M+K<sup>+</sup>]<sup>+</sup>.

TLC (SiO<sub>2</sub>, EtOH—conc. NH<sub>4</sub>OH 2:1): R<sub>f</sub> = 0.7.

10 EA (C<sub>19</sub>H<sub>35</sub>N<sub>7</sub>O<sub>4</sub>P<sub>2</sub>·0.5TFA·1.5H<sub>2</sub>O, M<sub>R</sub> = 662.9): C 36.2 (36.4); H 5.9 (5.6); N 14.8 (15.0).

Example 14: *Synthesis of intermediate product C*



15 In a glass flask (100 mL), methylene-bis(phosphinic acid) (2.32 g; 16.1 mol; 1.0 equiv.) and dibenzylamine (4.03 mL; 21.0 mmol; 1.3 equiv.) were suspended in a mixture of 6 M aqueous HCl—THF (1:1; 50 mL). Paraformaldehyde (726 mg; 24.2 mmol; 1.5 equiv.) was then added, the flask was quickly sealed with stopper and the resulting mixture was stirred at 80 °C overnight. After cooling to room temperature, the mixture  
 20 was evaporated to dryness. The residue was dissolved in H<sub>2</sub>O and washed with CH<sub>2</sub>Cl<sub>2</sub>. Aqueous layer was separated, evaporated to dryness and the residue was purified was purified by flash chromatography (C18; gradient elution H<sub>2</sub>O—0,1% TFA—MeCN). Fraction with product was evaporated to dryness and the residue was re-dissolved in small volume of MeOH. Et<sub>2</sub>O was subsequently added until cloudiness. Next day, the  
 25 formed precipitate was collected on glass frit S3 and washed with Et<sub>2</sub>O. Product was obtained as a white substance (2.27 mg; 37%; 1 step; based on methylene-bis(phosphinic acid)).

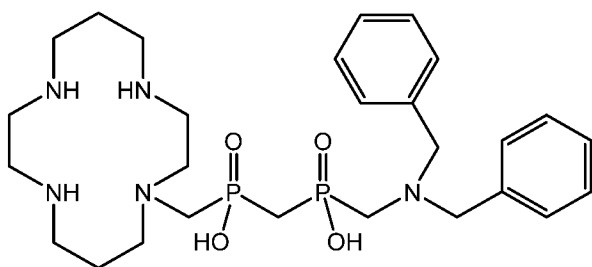
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 2.02 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz); 3.25 (P—CH<sub>2</sub>—N, d, 2H, <sup>2</sup>J<sub>HP</sub> = 11 Hz); 4.46 (CH<sub>2</sub>—Ph, m, 4H); 7.15 (PH, d, 1H, <sup>1</sup>J<sub>HP</sub> = 562 Hz); 7.52 (Ph, m, 10H); <sup>13</sup>C{<sup>1</sup>H} δ 35.8 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 81 Hz); 51.7 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 92 Hz); 59.4 (CH<sub>2</sub>—Ph, s); 129.8 (C—CH<sub>2</sub>, s); 129.9 (Ph, s); 130.8 (Ph, s); 132.1 (Ph, s); <sup>31</sup>P δ 20.9 (P—CH<sub>2</sub>—N, m, 1P); 22.1 (PH, dm, 1P, <sup>1</sup>J<sub>PH</sub> = 562 Hz).

**MS:** (–) 351.6 [M–H<sup>+</sup>]<sup>–</sup>. (+) 353.7 [M+H<sup>+</sup>]<sup>+</sup>; 391.7 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, MeOH): R<sub>f</sub> = 0.6.

**EA** (C<sub>16</sub>H<sub>21</sub>NO<sub>4</sub>P<sub>2</sub>·MeOH, M<sub>R</sub> = 385.3): C 53.0 (53.1); H 6.5 (6.2); N 3.6 (3.9).

10 Example 15: *Synthesis of compound 12*



To a glass flask (100 ml), 1,4,8,11-tetraazacyclotetradecane (3.26 g; 16.3 mmol; 5.5 equiv.) was added. Intermediate C·MeOH (1.15 g; 2.99 mmol; 1.0 equiv.), paraformaldehyde (160 mg; 5.33 mmol; 1.8 equiv.) and aqueous HCl (6 M; 60 mL) were subsequently added and the resulting mixture was stirred at 80 °C overnight. Mixture was evaporated to dryness and co-evaporated several times with H<sub>2</sub>O. Crude product was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 5% aqueous ammonia). Ammonia fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 1; OH<sup>–</sup>-form; H<sub>2</sub>O → 10% aqueous AcOH). Acetate fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was further dried on vacuum pump and subsequently in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. Product was obtained as a white substance (1.46 g; 74%; 1 step, based on intermediate C·MeOH).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.67 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 5 Hz); 1.71 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 5 Hz); 2.07 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.55–2.73 (N—CH<sub>2</sub>—P, cycle, m, 18H); 2.76 (N—CH<sub>2</sub>—P, d, 2H, <sup>2</sup>J<sub>HP</sub> = 9 Hz); 3.79 (CH<sub>2</sub>—Ph, s, 4H); 7.32–7.49 (Ph, m, 10H); <sup>13</sup>C{<sup>1</sup>H} δ 25.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 27.0 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.5 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 77 Hz); 45.7 (cycle, s); 45.9 (cycle, s); 46.3

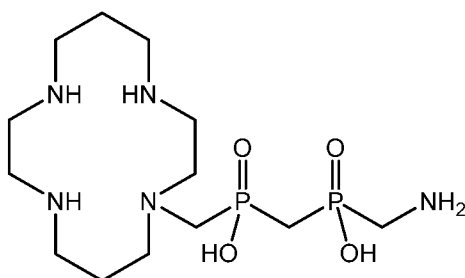
(*cycle*, s); 46.4 (*cycle*, s); 53.7 (*cycle*, s); 54.6 (*cycle*, s); 54.9 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 107 Hz); 55.2 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); 58.9 (CH<sub>2</sub>—Ph, d, <sup>3</sup>J<sub>CP</sub> = 6 Hz); 127.8 (*Ph*, s); 128.9 (*Ph*, s); 130.3 (*Ph*, s); 139.0 (*Ph*, s); <sup>31</sup>P{<sup>1</sup>H} δ 33.1 (P—CH<sub>2</sub>—N, d, 1P, <sup>2</sup>J<sub>PP</sub> = 12 Hz) 33.6 (P—CH<sub>2</sub>—N, d, 1P, <sup>2</sup>J<sub>PP</sub> = 12 Hz).

5 **MS**: (–) 563.8 [M–H<sup>+</sup>]<sup>–</sup>. (+) 566.0 [M+H<sup>+</sup>]<sup>+</sup>; 588.0 [M+Na<sup>+</sup>]<sup>+</sup>; 604.0 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 2:1): R<sub>f</sub> = 0.8.

**EA** (C<sub>27</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>P<sub>2</sub>·AcOH·1.5H<sub>2</sub>O, M<sub>R</sub> = 657.2): C 53.4 (53.4); H 8.0 (7.7); N 10.7 (10.6).

10 **Example 16: Synthesis of compound 13**



In a glass flask (250 mL), **12**·AcOH·1.5H<sub>2</sub>O (1.55 g; 2.36 mmol) was dissolved in H<sub>2</sub>O and the resulting solution was briefly washed with argon gas. HCOONH<sub>4</sub> (4.45 g; 70.5 mmol; 30 equiv.) and catalyst (10% Pd@C; 113 mg) were subsequently added and the resulting suspension was stirred at 60 °C for three hours. Another portion of HCOONH<sub>4</sub> (1.48 g; 23.5 mmol; 10 equiv.) and catalyst (10% Pd@C; 41 mg) was added and the mixture was further stirred for two days. The mixture was evaporated to dryness and further co-evaporated several times with H<sub>2</sub>O. The residue was dissolved in H<sub>2</sub>O, catalyst was filtered off and the filtrate was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 5% aqueous ammonia). Ammonia fraction with product was evaporated to dryness and further co-evaporated several times with H<sub>2</sub>O. The residue was re-dissolved in H<sub>2</sub>O (150 ml) and lyophilized. Product was obtained as an off-white substance (720 mg; 74%; 1 step; based on **13**·AcOH·1.5H<sub>2</sub>O).

25 **NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.69 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 6 Hz); 1.81 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.11 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.61–3.32 (N—CH<sub>2</sub>—P, *cycle*, m, 20H); <sup>13</sup>C{<sup>1</sup>H} δ 29.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 30.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 36.4 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 78 Hz); 39.9 (*cycle*, s); 42.2 (*cycle*, s); 43.3 (*cycle*, s); 44.3 (*cycle*, s); 45.8 (*cycle*, s); 51.1 (*cycle*, s); 51.8 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> =

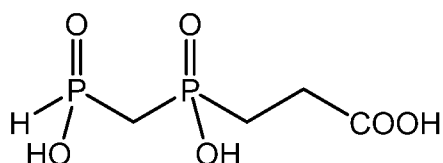
105 Hz); 57.8 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 102 Hz); <sup>31</sup>P{<sup>1</sup>H} δ 24.0 (P—CH<sub>2</sub>—NH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 5 Hz); 31.3 (C—N—CH<sub>2</sub>—P, d, 1P, <sup>2</sup>J<sub>PP</sub> = 5 Hz).

MS: (-) 383.9 [M—H<sup>+</sup>]<sup>-</sup>. (+) 385.7 [M+H<sup>+</sup>]<sup>+</sup>; 407.9 [M+Na<sup>+</sup>]<sup>+</sup>.

TLC (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.6.

5 EA (C<sub>13</sub>H<sub>33</sub>N<sub>5</sub>O<sub>4</sub>P<sub>2</sub>·2.5H<sub>2</sub>O, M<sub>R</sub> = 412.4): C 40.5 (40.3); H 8.6 (8.9); N 18.2 (18.4).

Example 17: *Synthesis of intermediate D*



10 In a argon-washed three-necked flask (1000 mL), methylene-bis(phosphinic acid) (2.50 g; 17.4 mol; 1.0 equiv.) was suspended in hexamethyldisilazane (150 mL; 716 mmol; 41 equiv.) and the resulting mixture was stirred at 110 °C under gentle flow of argon overnight. The mixture was then cooled to room temperature and dry CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was subsequently added followed by addition of *tert*-butyl acrylate (5.00 mL; 34.2 mmol; 2.0 equiv.) in dry CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The resulting mixture was further stirred at  
15 room temperature overnight. Reaction was quenched with EtOH (150 mL) and the resulting mixture was evaporated to dryness. The crude product was purified by flash chromatography (C18; gradient elution H<sub>2</sub>O–0,1% TFA–MeCN). Fractions with pure product were combined and evaporated to dryness. The residue was further purified by ion exchange chromatography (Amberlite CG50; H<sup>+</sup>-form; H<sub>2</sub>O). Aqueous fraction  
20 was evaporated to dryness and further co-evaporated several times with CH<sub>2</sub>Cl<sub>2</sub>. Residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>–TFA (1:1; 200 mL) and the resulting mixture was stirred overnight at RT in the absence of light. Reaction mixture was then evaporated to dryness and the residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were combined and evaporated to dryness. The  
25 residue was further purified on vacuum pump and subsequently in vacuum dessicator over P<sub>2</sub>O<sub>5</sub>. Product was obtained as a white substance (1.41 g; 38%; 2 steps; based on methylene-bis(phosphinic acid)).

30 NMR (D<sub>2</sub>O): <sup>1</sup>H δ 1.97 (P—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.21 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz); 2.46 (P—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 7.01 (PH, 1H, d, <sup>1</sup>J<sub>HP</sub> = 560 Hz); <sup>13</sup>C{<sup>1</sup>H} 26.2 (P—CH<sub>2</sub>—CH<sub>2</sub>, d, <sup>1</sup>J<sub>CP</sub> = 98 Hz); 27.1 (P—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.0 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> =

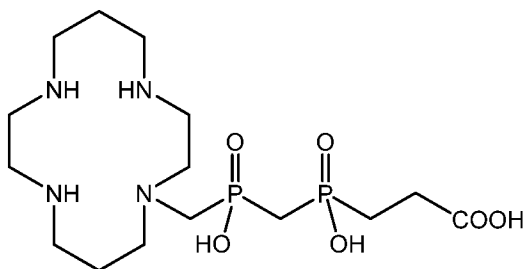
79 Hz); 177.2 (CO, d,  $^3J_{CP} = 16$  Hz);  $^{31}\text{P}$   $\delta$  19.2 (PH, d, 1P,  $^1J_{PH} = 544$  Hz); 44.5 (P—CH<sub>2</sub>—CH<sub>2</sub>, m, 1P).

MS: (–) 196.6 [M–H<sub>3</sub>O<sup>+</sup>]<sup>–</sup>; 214.5 [M–H<sup>+</sup>]<sup>–</sup>. (+) 216.7 [M+H<sup>+</sup>]<sup>+</sup>.

TLC (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 1:1): *R<sub>f</sub>* = 0.6.

5 EA (C<sub>4</sub>H<sub>10</sub>O<sub>6</sub>P<sub>2</sub>, *M<sub>R</sub>* = 216.1): C 22.2 (21.9); H 4.7 (5.0).

Example 18: *Synthesis of compound 14*



To a glass vial (20 ml), 1,4,8,11-tetraazacyclotetradecane (1.19 g; 5.95 mmol; 3.8 equiv.) was added. Intermediate **D** (340 mg; 1.57 mmol; 1.0 equiv.), paraformaldehyde (58 mg; 1.3 mmol; 1.2 equiv.) and aqueous HCl (6 M; 10 mL) were subsequently added and the resulting mixture was stirred at 60 °C overnight. Next day, mixture was evaporated to dryness and co-evaporated several times with H<sub>2</sub>O. Crude product was purified by ion exchange chromatography (IRA 402; OH<sup>–</sup>-form; H<sub>2</sub>O → 10% aqueous AcOH). Acetate fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). Pyridine fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was re-dissolved in H<sub>2</sub>O (50 mL) and subsequently lyophilized. Product was obtained as a white substance (459 mg; 61%; 1 step, based on intermediate **D**).

NMR (D<sub>2</sub>O):  $^1\text{H}$   $\delta$  1.71 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H,  $^3J_{\text{HH}} = 5$  Hz); 1.76 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H,  $^3J_{\text{HH}} = 5$  Hz); 1.91 (P—CH<sub>2</sub>—C, m, 2H); 2.05 (P—CH<sub>2</sub>—P, t, 2H,  $^2J_{\text{HP}} = 16$  Hz); 2.37 (CH<sub>2</sub>—CO, m, 2H); 2.65 (*cycle*, t, 2H,  $^3J_{\text{HH}} = 5$  Hz); 2.69–2.79 (*cycle*, N—CH<sub>2</sub>—P, m, 16H);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  25.6 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 27.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 29.7 (P—CH<sub>2</sub>—C, d,  $^1J_{\text{CP}} = 96$  Hz); 31.2 (CH<sub>2</sub>—CO, s); 33.5 (P—CH<sub>2</sub>—P, t,  $^1J_{\text{CP}} = 77$  Hz); 46.2 (*cycle*, s); 46.3 (*cycle*, s); 46.8 (*cycle*, s); 46.9 (*cycle*, s); 47.6 (*cycle*, s); 48.8 (*cycle*, s); 54.4 (*cycle*, s); 54.8 (*cycle*, s); 55.2 (N—CH<sub>2</sub>—P, d,  $^1J_{\text{CP}} = 109$

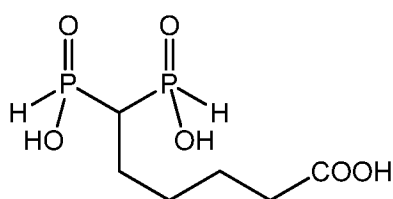
Hz); 183.2 (CO, d,  $^3J_{CP} = 19$  Hz);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  33.4 (P—CH<sub>2</sub>—N, d, 1P,  $^2J_{PP} = 10$  Hz); 36.6 (P—CH<sub>2</sub>—CH<sub>2</sub>, d, 1P,  $^2J_{PP} = 10$  Hz).

MS: (-) 402.8 [M—H<sub>3</sub>O<sup>+</sup>]<sup>-</sup>; 426.7 [M—H<sup>+</sup>]<sup>-</sup>. (+) 428.9 [M+H<sup>+</sup>]<sup>+</sup>; 450.8 [M+Na<sup>+</sup>]<sup>+</sup>; 466.8 [M+K<sup>+</sup>]<sup>+</sup>.

5 TLC (SiO<sub>2</sub>, *i*PrOH–conc.NH<sub>4</sub>OH–H<sub>2</sub>O 7:1:1):  $R_f = 0.5$ .

EA (C<sub>15</sub>H<sub>34</sub>N<sub>4</sub>O<sub>6</sub>P<sub>2</sub>·4H<sub>2</sub>O,  $M_R = 500.5$ ): C 36.0 (36.0); H 8.5 (8.8); N 11.2 (11.2).

Example 19: *Synthesis of intermediate product E*



10 In a glass beaker (1000 mL), NaH<sub>2</sub>PO<sub>2</sub>·H<sub>2</sub>O (28.6 g; 270 mmol; 6.0 equiv.) was dissolved in a mixture of MeOH (500 mL) and unstabilized dioxane (75 mL). 5-hexynoic acid (5.00 mL; 45.3 mmol; 1.0 equiv.) and solution of Et<sub>3</sub>B in hexanes (1.0 M; 45 mL; 45.0 mmol; 1.0 equiv.) were subsequently added and the resulting mixture was vigorously stirred in presence of air for four hours. The resulting precipitate was

15 collected on glass frit S3 and several times washed with cold MeOH and air dried. The crude product was purified by column chromatography (SiO<sub>2</sub>; EtOH–conc. NH<sub>4</sub>OH 2:1;  $R_f = 0.4$ ). Fractions with product were combined and evaporated to dryness. The residue was re-dissolved in H<sub>2</sub>O (150 mL) and treated with charcoal. The resulting suspension was filtered through syringe microfilter (Millipore; 0.22

20  $\mu\text{m}$ ) and the filtrate was further purified by ion exchange resin (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Aqueous fraction was evaporated to dryness, re-dissolved in H<sub>2</sub>O and subsequently lyophilized (4.31 g; 32%; 1 step; based on Et<sub>3</sub>B).

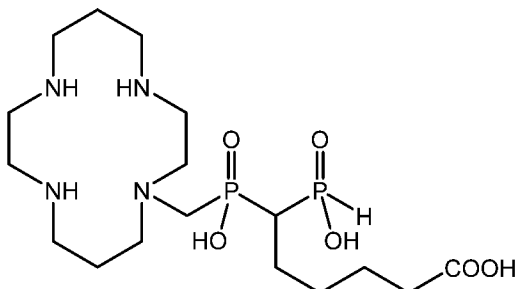
NMR (D<sub>2</sub>O):  $^1\text{H}$   $\delta$  1.57 (CH<sub>2</sub>, m, 2H); 1.64 (CH<sub>2</sub>, m, 2H); 1.74 (CH<sub>2</sub>, m, 2H); 1.87 (P—CH—P, m, 1H); 2.38 (CH<sub>2</sub>—CO, t, 2H,  $^3J_{HH} = 8$  Hz); 7.04 (PH, dm, 2H,  $^1J_{HP} = 526$  Hz);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  21.7 (P—CH—CH<sub>2</sub>—CH<sub>2</sub>, t,  $^3J_{CP} = 3$  Hz); 25.6 (CH<sub>2</sub>—CH<sub>2</sub>—CO, s); 29.4 (P—CH—CH<sub>2</sub>, t,  $^2J_{CP} = 7$  Hz); 35.2 (CH<sub>2</sub>—CO, s); 44.8 (P—CH—P, t,  $^1J_{CP} = 78$  Hz); 180.9 (CO, s);  $^{31}\text{P}$   $\delta$  25.8 (dm,  $^1J_{PH} = 526$  Hz).

MS: (-) 242.5 [M—H<sup>+</sup>]<sup>-</sup>; 264.5 [M—2H<sup>+</sup>+Na<sup>+</sup>]<sup>-</sup>; 286.5 [M—3H<sup>+</sup>+2Na<sup>+</sup>]<sup>-</sup>. (+) 310.8 [M—2H<sup>+</sup>+3Na<sup>+</sup>]<sup>+</sup>.

30 TLC (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 2:1):  $R_f = 0.4$ .

EA (C<sub>6</sub>H<sub>14</sub>O<sub>6</sub>P<sub>2</sub>·3H<sub>2</sub>O, M<sub>R</sub> = 298.2): C 24.2 (24.1); H 6.8 (6.5).

Example 20: *Synthesis of compound 15*



5 To a glass vial (20 ml), 1,4,8,11-tetraazacyclotetradecane (1.50 g; 7.50 mmol; 3.8 equiv.) was added. Intermediate E·3H<sub>2</sub>O (1.51 g; 5.08 mmol; 2.6 equiv.), paraformaldehyde (59.2 mg; 1.97 mmol; 1.0 equiv.) and aqueous HCl (6 M; 10 mL) were subsequently added and the resulting mixture was stirred at 70 °C overnight. Next day, the mixture was evaporated to dryness and co-evaporated several times  
10 with H<sub>2</sub>O. The crude product was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). Pyridine fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The crude product was purified by column chromatography (SiO<sub>2</sub>; *i*PrOH-konc. NH<sub>4</sub>OH-H<sub>2</sub>O 7:3:3; R<sub>f</sub> = 0.5) Fractions with product were combined and evaporated  
15 to dryness. The residue was suspended in H<sub>2</sub>O (50 mL) and treated with charcoal. Suspension was then filtered through syringe microfilter (Millipore; 0.22 μm). The filtrate was re-purified by ion exchange chromatography (IRA 402; OH<sup>-</sup>-form; H<sub>2</sub>O → 10% aqueous AcOH). Acetate fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was re-dissolved in H<sub>2</sub>O (50 mL)  
20 and subsequently lyophilized. Product was obtained as a white substance (621 mg; 61%; 1 step, based on paraformaldehyde).

**NMR (D<sub>2</sub>O):** <sup>1</sup>H δ 1.39 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, m, 1H); 1.50 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, m, 3H); 1.57-1.77 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, CH<sub>2</sub>-CH-P, P-CH-P, m, 7H); 2.12 (CH<sub>2</sub>-COOH, t, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.51-2.79 (*cycle*, N-CH<sub>2</sub>-P, m, 18H); 7.05 (PH, d, <sup>1</sup>J<sub>HP</sub> = 525 Hz); <sup>13</sup>C {<sup>1</sup>H} δ 23.8 (CH<sub>2</sub>-CH<sub>2</sub>-CH-P, t, <sup>3</sup>J<sub>CP</sub> = 3 Hz); 25.6 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, s); 26.9 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, s); 27.2 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, s); 30.8 (CH<sub>2</sub>-CH-P, dd, <sup>2</sup>J<sub>CP</sub> = 8 Hz, <sup>2</sup>J<sub>CP</sub> = 4 Hz); 38.3 (CH<sub>2</sub>-CO, s); 45.0 (P-CH-P, dd, <sup>1</sup>J<sub>CP</sub> = 79 Hz, <sup>1</sup>J<sub>CP</sub> = 73 Hz); 45.8 (*cycle*, s); 46.1 (*cycle*, s); 46.4 (*cycle*, s); 46.7 (*cycle*, s); 47.3 (*cycle*, s);

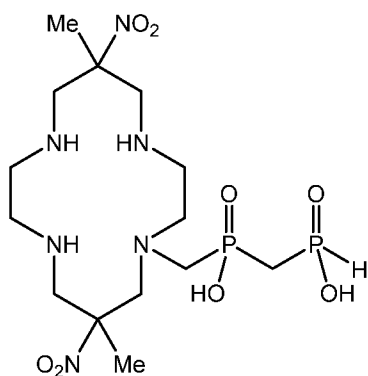
48.2 (*cycle*, s); 53.3 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 103 Hz); 54.1 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 7 Hz); 54.7 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 6 Hz); 184.6 (CO, s); <sup>31</sup>P δ 25.7 (PH, dm, 1P, <sup>1</sup>J<sub>HP</sub> = 525 Hz); 36.3 (P—CH<sub>2</sub>—N, m, 1P).

MS: (-) 454.7 [M-H<sup>+</sup>]<sup>-</sup>. (+) 457.0 [M+H<sup>+</sup>]<sup>+</sup>.

5 TLC (SiO<sub>2</sub>, *i*PrOH–conc. NH<sub>4</sub>OH–H<sub>2</sub>O 7:1:1): R<sub>f</sub> = 0.5.

EA (C<sub>14</sub>H<sub>32</sub>N<sub>4</sub>O<sub>5</sub>P<sub>2</sub>·AcOH, M<sub>R</sub> = 516.5): C 44.2 (44.1); H 8.2 (8.5); N 10.9 (10.7).

Example 21: *Synthesis of compound 16*



10 To a glass flask (25 ml), 6,13-dimethyl-6,13-dinitro-1,4,8,11-tetraazacyclotetradecane (411 mg; 885 mmol; 4.7 equiv.) was added. Methylene-bis(phosphonic acid) (61.3 mg; 426 μmol; 2.3 equiv.) and paraformaldehyde (5.6 mg; 187 μmol; 1.0 equiv.) and aqueous HCl (6 M; 10 mL) were subsequently added and the resulting mixture was stirred at 60 °C for two days. The mixture was then  
 15 evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The crude product was purified by ion exchange chromatography (DOWEX 50; 200 mL; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). Pyridine fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was further dried on vacuum pump and in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. Product was obtained as a yellowish  
 20 substance (92.2 mg; 82%; 1 step, based on methylene-bis(phosphonic acid)).

NMR (D<sub>2</sub>O): <sup>1</sup>H δ 1.65 (CH<sub>3</sub>, s, 3H); 1.74 (CH<sub>3</sub>, s, 3H); 2.12 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 18 Hz, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.69–2.94 (*cycle*, N—CH<sub>2</sub>—P m, 8H); 3.03–3.41 (*cycle*, m, 10H); 7.14 (PH, d, 1H, <sup>1</sup>J<sub>HP</sub> = 530 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 24.2 (CH<sub>3</sub>, s); 25.2 (CH<sub>3</sub>, s); 35.5 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 79 Hz, <sup>1</sup>J<sub>CP</sub> = 76 Hz); 48.2 (*cycle*, s); 48.8 (*cycle*, s);  
 25 49.2 (*cycle*, s); 50.1 (*cycle*, s); 52.3 (*cycle*, s); 52.4 (*cycle*, s); 55.2 (*cycle*, s); 57.6 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 104 Hz); 58.2 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 10 Hz); 89.9 (C—NO<sub>2</sub>, s); 91.8

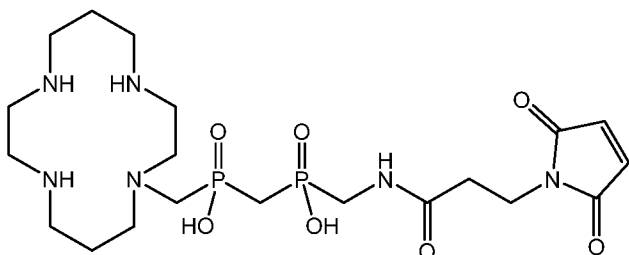
(C—NO<sub>2</sub>, s); <sup>31</sup>P δ 16.2 (PH, dt, 1P, <sup>1</sup>J<sub>PH</sub> = 530 Hz, <sup>2</sup>J<sub>PH</sub> = 18 Hz); 36.9 (N—CH<sub>2</sub>—P, m, 1P).

MS: (–) 473.3 [M–H<sup>+</sup>]<sup>–</sup>. (+) 475.1 [M+H<sup>+</sup>]<sup>+</sup>; 513.4 [M+K<sup>+</sup>]<sup>+</sup>.

TLC (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.8.

5 EA (C<sub>14</sub>H<sub>32</sub>N<sub>6</sub>O<sub>8</sub>P<sub>2</sub>·3HCl·H<sub>2</sub>O, M<sub>R</sub> = 601.8): C 27.9 (27.7); H 6.2 (6.5); N 14.0 (14.3).

Example 22: *Synthesis of conjugate 17*



10 In a glass vial (4 ml), **13**·1.5H<sub>2</sub>O (14.1 mg; 34.2 μmol; 1.0 equiv.) and *N*-hydroxysuccinimide ester of 3-(maleimido)propanoic acid (10.0 mg; 37.6 μmol; 1.1 equiv.) were dissolved in aqueous buffer H<sub>3</sub>PO<sub>4</sub>–NaOH (1.0 M pH = 8.1; 1.00 mL; 1.0 mmol; 29 equiv.). The resulting mixture was stirred at room temperature overnight. The crude product was purified by preparative HPLC (C8; H<sub>2</sub>O–MeCN). Fraction with product was lyophilized. Product was obtained as a white substance (13.4 mg; 15 45%; 1 step, based on **13**·1.5H<sub>2</sub>O).

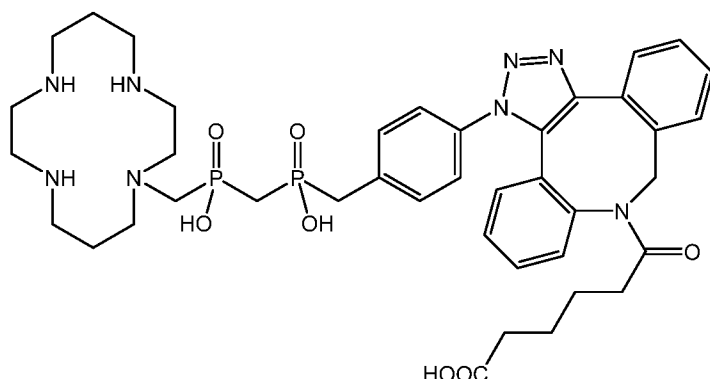
NMR (D<sub>2</sub>O): <sup>1</sup>H δ 1.99 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.12 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.18 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.48–3.11 (CH<sub>2</sub>—CO, N—CH<sub>2</sub>—P, cycle, m, 20H); 4.21 (CH<sub>2</sub>—N—CO, t, <sup>3</sup>J<sub>HH</sub> = 9 Hz); 6.82 (CH, s, 2H); <sup>13</sup>C{<sup>1</sup>H} δ 22.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 23.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 29.8 (CH<sub>2</sub>—CO, s); 20 34.1 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 79 Hz); 43.1 (CH<sub>2</sub>—N—CO, s); 41.9 (cycle, s); 43.6 (cycle, s); 43.9 (cycle, s); 44.3 (cycle, s); 46.8 (cycle, s); 47.2 (cycle, s); 53.4 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 96 Hz); 53.6 (cycle, s); 55.1 (cycle, s); 55.2 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 108 Hz); 133.7 (CH, s); 169.8 (CO—N—CO, s); 174.5 (CH<sub>2</sub>—CO—N, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz). <sup>31</sup>P{<sup>1</sup>H} δ 32.8 (P—CH<sub>2</sub>—N, d, 1P, <sup>2</sup>J<sub>PP</sub> = 11 Hz); 35.2 (P—CH<sub>2</sub>—N, d, 1P, <sup>2</sup>J<sub>PP</sub> = 11 Hz). 25

MS: (–) 535.0 [M–H<sup>+</sup>]<sup>–</sup>. (+) 537.1 [M+H<sup>+</sup>]<sup>+</sup>; 559.1 [M+Na<sup>+</sup>]<sup>+</sup>; 575.1 [M+K<sup>+</sup>]<sup>+</sup>.

TLC (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 2:1): R<sub>f</sub> = 0.5.

EA (C<sub>20</sub>H<sub>38</sub>N<sub>6</sub>O<sub>7</sub>P<sub>2</sub>·TFA·2H<sub>2</sub>O, M<sub>R</sub> = 869.3): C 30.4 (30.7); H 5.0 (4.7); N 9.7 (9.5).

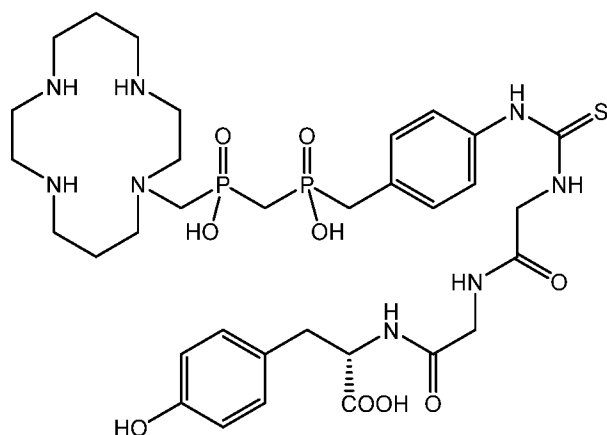
## Example 23: Synthesis of conjugate 18



To a glass vial (4 mL), solution of  $11 \cdot 0.5\text{TFA} \cdot 1.5\text{H}_2\text{O}$  (20.4 mM in  $\text{D}_2\text{O}$ ; 420  $\mu\text{L}$ ; 8.57  $\mu\text{mol}$ ; 1.0 equiv.) was pipetted and, then, freshly prepared solution of 6-dibenzocyclooctynamidexanoic acid (3.0 mg; 9.0  $\mu\text{mol}$ ; 1.1 equiv.) in a mixture of dry DMSO–dry MeCN (1:1; 500  $\mu\text{L}$ ) was added. The resulting mixture was stirred at room temperature for 2 hours. The crude product was purified by preparative HPLC (C8;  $\text{H}_2\text{O}$ –MeCN). Fractions with product were lyophilized. Product as two regioisomers was obtained as a white substance ( $2 \times \sim 3$  mg).

**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.18 ( $\text{CH}_2$ , m, 2H); 1.32 ( $\text{CH}_2$ , m, 2H); 2.04 (*cycle*, p,  $^3J_{\text{HH}} = 6$  Hz); 2.15 (*cycle*, P– $\text{CH}_2$ –P,  $\text{CH}_2$ , m, 6H); 2.36 ( $\text{CH}_2$ , m, 1H); 2.56 ( $\text{CH}_2$ , m, 1H); 2.67–3.63 (*cycle*, N– $\text{CH}_2$ –P, P– $\text{CH}_2$ –C, m, 20H); 5.07 ( $\text{CH}_2$ –N–CO, d, 1H,  $^2J_{\text{HH}} = 18$  Hz); 5.67 ( $\text{CH}_2$ –N–CO, d, 1H,  $^2J_{\text{HH}} = 18$  Hz); 6.89 ( $\text{CH}$ , d, 1H,  $^3J_{\text{HH}} = 8$  Hz); 7.21 ( $\text{CH}$ , t, 1H,  $^3J_{\text{HH}} = 8$  Hz); 7.29 ( $\text{CH}$ , d, 1H,  $^3J_{\text{HH}} = 8$  Hz); 7.39 ( $\text{CH}$ , m, 1H); 7.42 ( $\text{CH}$ , m, 1H); 7.45 ( $\text{CH}$ –C– $\text{CH}_2$ –P, d, 2H,  $^3J_{\text{HH}} = 8$  Hz); 7.50 ( $\text{CH}$ –C– $\text{N}_3$ ,  $\text{CH}$ , m, 3H); 7.55 ( $\text{CH}$ , m, 2H);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  23.0 (*cycle*, s); 23.3 (*cycle*, s); 24.3 ( $\text{CH}_2$ , s); 24.6 ( $\text{CH}_2$ , s); 32.4 (P– $\text{CH}_2$ –P, t,  $^1J_{\text{CP}} = 80$  Hz); 33.3 ( $\text{CH}_2$ , s); 34.1 ( $\text{CH}_2$ , s); 40.2 (P– $\text{CH}_2$ –P, d,  $^1J_{\text{CP}} = 89$  Hz); 42.9 (*cycle*, s); 44.2 (*cycle*, s); 44.9 (*cycle*, s); 45.4 (*cycle*, s); 46.0 (*cycle*, s); 46.4 (*cycle*, s); 53.6 (N– $\text{CH}_2$ –P, d,  $^1J_{\text{CP}} = 106$  Hz); 55.0 (*cycle*, s); 55.9 (*cycle*, d,  $^3J_{\text{CP}} = 11$  Hz); 55.9 ( $\text{CH}_2$ –N–CO, s); 116.1–145.2 ( $4 \times \text{arom.}$ ,  $14 \times \text{arom.}$ ); 177.1 (CO–N, s); 178.9 (CO–O, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  30.9 ( $\text{CH}_2$ –P– $\text{CH}_2$ , m, 1P); 34.5 ( $\text{CH}_2$ –P– $\text{CH}_2$ , d, 1P,  $^2J_{\text{PP}} = 10$  Hz).

**MS:** (–) 818.8  $[\text{M}-\text{H}]^-$ . (+) 821.1  $[\text{M}+\text{H}]^+$ ; 843.0  $[\text{M}+\text{Na}]^+$ ; 859.0  $[\text{M}+\text{K}]^+$ .

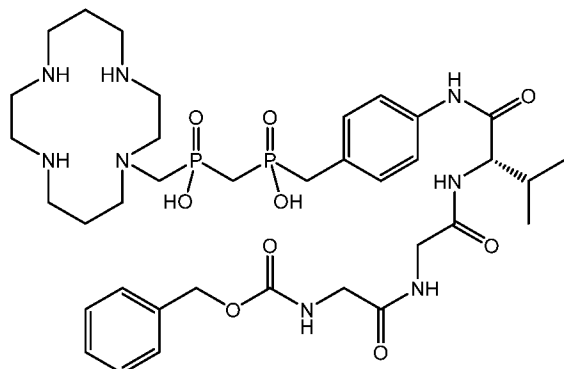
Example 24: Synthesis of targeting conjugate **19**

In an NMR tube, **10**·TFA·3.5H<sub>2</sub>O (3.4 mg; 3.9 μmol; 1.0 equiv.) was dissolved in D<sub>2</sub>O (500 μL). The HGlyGlyTyrOH (1.9 mg; 6.2 μmol; 1.6 equiv.) and aqueous buffer H<sub>3</sub>BO<sub>3</sub>-LiOH (750 mM in D<sub>2</sub>O; pD = 9.3; 413 μL; 310 μmol; 80 equiv.) were then added and the mixture was stirred at room temperature overnight. The crude product was purified by preparative HPLC (C8; H<sub>2</sub>O—MeCN). Fraction with product was lyophilized. Product was obtained as a white substance (~1 mg).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 2.01 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, m, 2H); 2.06 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, m, 2H); 2.12 (P-CH<sub>2</sub>-P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.58-3.56 (*cycle*, N-CH<sub>2</sub>-P, P-CH<sub>2</sub>-C, CH<sub>2</sub>, m, 22H); 3.90 (CH<sub>2</sub>, m, 2H); 4.21 (CH<sub>2</sub>, m, 2H); 4.64 (CH-N, dd, 1H, <sup>3</sup>J<sub>HH</sub> = 9 Hz, <sup>3</sup>J<sub>HH</sub> = 5 Hz); 6.85 (CH-C-OH, d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); 7.16 (CH-CH-CH<sub>2</sub>, d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); 7.32 (CH-C-NH, d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); 7.38 (CH-C-CH<sub>2</sub>, d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 23.0 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, s); 23.8 (CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, s); 32.4 (P-CH<sub>2</sub>-P, t, <sup>1</sup>J<sub>CP</sub> = 80 Hz); 36.5 (CH<sub>2</sub>, s); 40.1 (P-CH<sub>2</sub>-C, d, <sup>1</sup>J<sub>CP</sub> = 89 Hz); 42.9 (CH<sub>2</sub>, s); 43.3 (*cycle*, s); 44.8 (*cycle*, s); 45.6 (*cycle*, s); 46.0 (*cycle*, s); 46.4 (*cycle*, s); 47.0 (*cycle*, s); 48.2 (CH<sub>2</sub>, s); 53.5 (P-CH<sub>2</sub>-N, d, <sup>1</sup>J<sub>CP</sub> = 107 Hz); 54.9 (*cycle*, s); 55.1 (CH-N, s); 55.8 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 11 Hz); 116.1 (CH-C-OH, s); 126.8 (CH-C-NH, s); 129.2 (C-CH<sub>2</sub>, s); 131.3 (CH-C-CH<sub>2</sub>, s); 131.5 (CH-C-CH<sub>2</sub>, s); 134.3 (C-CH<sub>2</sub>-P, d, <sup>2</sup>J<sub>CP</sub> = 6 Hz); 135.8 (C-NH, s); 155.1 (C-OH, s); 171.9 (CO, s); 173.1 (CO, s); 175.7 (CO, s); 182.3 (CS, s); <sup>31</sup>P{<sup>1</sup>H} δ 31.2 (N-CH<sub>2</sub>-P, m, 1P); 34.2 (P-CH<sub>2</sub>-C, d, 1P, <sup>2</sup>J<sub>PP</sub> = 8 Hz).

**MS**: (-) 796.8 [M-H<sup>+</sup>]<sup>-</sup>. (+) 799.0 [M+H<sup>+</sup>]<sup>+</sup>.

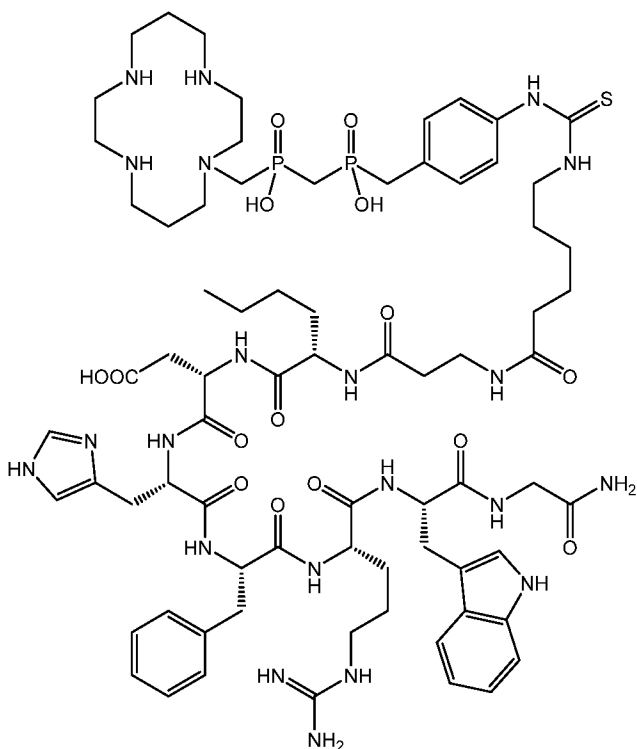
## Example 25: Synthesis of targeting conjugate 20



In a glass vial (4 mL), HOValGlyGlyCbz (9.6 mg; 26  $\mu\text{mol}$ ; 3.0 equiv.) was dissolved in  $\text{H}_2\text{O}$ -MeCN (2:3; 1.0 mL). Trichlorotriazine (1.6 mg; 8.7  $\mu\text{mol}$ ; 1.0 equiv.) and pyridine (21.2  $\mu\text{L}$ ; 0.26 mmol; 30 equiv.) were subsequently added and the resulting mixture was stirred at room temperature for one hour. Solution of  $\mathbf{9} \cdot 3\text{H}_2\text{O}$  (17.4 mg; 33.8  $\mu\text{mol}$ ; 3.9 equiv.) in a mixture of  $\text{H}_2\text{O}$ -MeCN (2:3; 1.0 mL) was then added and the resulting mixture was further stirred at room temperature overnight. The crude product was purified by preparative HPLC (C8;  $\text{H}_2\text{O}$ -MeCN). Fraction with product was lyophilized. Product was obtained as a white substance (~1 mg).

**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.02 ( $\text{CH}_3$ , m, 6H); 2.02 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ , p, 2H,  $^3J_{\text{HH}} = 6$  Hz); 2.07 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ , p, 2H,  $^3J_{\text{HH}} = 6$  Hz); 2.16 (P- $\text{CH}_2$ -P, t, 2H,  $^2J_{\text{HP}} = 16$  Hz); 2.20 ( $\text{CH}_3\text{-CH-CH}_3$ , m, 1H); 2.34-3.52 (*cycle*, N- $\text{CH}_2$ -P, P- $\text{CH}_2$ -C, bm, 20H); 3.91 ( $\text{CH}_2$ , s, 2H); 4.02 ( $\text{CH}_2$ , s, 2H); 4.23 (CH-CO, d, 1H,  $^3J_{\text{HH}} = 8$  Hz); 5.15 (Ph- $\text{CH}_2$ -O, s, 2H); 7.29-7.51 (CH-C-N, CH-C- $\text{CH}_2$ , Ph, bm, 9H);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  18.5 ( $\text{CH}_3$ , s); 19.1 ( $\text{CH}_3$ , s); 23.0 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ , s); 23.6 ( $\text{CH}_2\text{-CH}_2\text{-CH}_2$ , s); 31.0 ( $\text{CH}_3\text{-CH-CH}_3$ , s); 32.5 (P- $\text{CH}_2$ -P, t,  $^1J_{\text{CP}} = 80$  Hz); 40.0 (P- $\text{CH}_2$ -C, d,  $^1J_{\text{CP}} = 90$  Hz); 43.1 ( $\text{CH}_2$ , s); 43.2 (*cycle*, s); 44.5 ( $\text{CH}_2$ , s); 44.6 (*cycle*, s); 45.4 (*cycle*, s); 45.6 (*cycle*, s); 46.3 (*cycle*, s); 46.6 (*cycle*, s); 53.5 (P- $\text{CH}_2$ -N, d,  $^1J_{\text{CP}} = 107$  Hz); 54.8 (*cycle*, s); 55.8 (*cycle*, d,  $^3J_{\text{CP}} = 10$  Hz); 61.1 (CH-CO, s); 68.1 (Ph- $\text{CH}_2$ -O, s); 123.3 (Ph, s); 128.5 (CH-C-NH, s); 129.2 (Ph, s); 129.6 (Ph, s); 131.2 (CH-C- $\text{CH}_2$ , d,  $^3J_{\text{CP}} = 6$  Hz); 132.5 (C- $\text{CH}_2$ -P, d,  $^2J_{\text{CP}} = 8$  Hz); 135.4 (C-NH, s); 137.0 (Ph, s); 159.4 (CO-O, s); 172.3 (CO, s); 173.0 (CO, s); 173.9 (CO, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  30.1 (N- $\text{CH}_2$ -P, m, 1P); 34.4 (P- $\text{CH}_2$ -C, d, 1P,  $^2J_{\text{PP}} = 9$  Hz).

**MS:** (-) 806.9 [ $\text{M-H}^+$ ] $^-$ . (+) 809.1 [ $\text{M+H}^+$ ] $^+$ ; 831.0 [ $\text{M+Na}^+$ ] $^+$ .

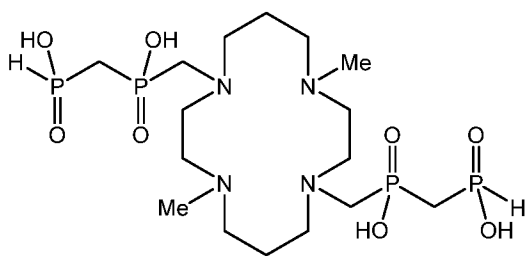
Example 26: Synthesis of targeting conjugate **21**

To a glass vial (4 mL), **10**·TFA·3.5H<sub>2</sub>O (13.2 mg; 18.5 μmol; 1.7 equiv.) was added. Peptide NH<sub>2</sub>(CH<sub>2</sub>)<sub>5</sub>CONH(CH<sub>2</sub>)<sub>2</sub>CONleAspHisD-PheArgTrpGlyNH<sub>2</sub> (12.0 mg; 10.8 μmol; 1.0 equiv.) and aqueous buffer H<sub>3</sub>BO<sub>3</sub>-LiOH (750 mM in D<sub>2</sub>O; pD = 9.3; 3.5 mL; 2.63 mmol; 240 equiv.) were subsequently added and the resulting mixture was stirred at room temperature overnight. The crude product was purified by preparative HPLC (C8; H<sub>2</sub>O—MeCN). Fraction with product was lyophilized. Product was obtained as a white substance (~2 mg).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 0.83 (CH<sub>3</sub>, t, 3H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 1.04 (CH<sub>2</sub>, bm, 2H); 1.21–1.72 (CH<sub>2</sub>, bm, 10H); 1.82–2.23 (P—CH<sub>2</sub>—P, CH<sub>2</sub>, cycle, bm, 8H); 2.35–3.54 (cycle, CH<sub>2</sub>, P—CH<sub>2</sub>—N, P—CH<sub>2</sub>—C, bm, 38H); 3.80 (CH<sub>2</sub>, m, 2H); 4.12 (CH, m, 1H); 4.16 (CH, m, 1H); 4.51 (CH, m, 1H); 4.55 (CH, m, 1H); 4.59 (CH, m, 1H); 4.69 (CH, m, 1H); 7.06 (CH<sub>2</sub>—C—CH—N, s, 1H); 7.10–7.27 (arom., m, 7H); 7.28–7.39 (arom., m, 5H); 7.46 (CH, d, 1H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 7.63 (CH, d, 1H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); 8.53 (N—CH—N, s, 1H); <sup>31</sup>P{<sup>1</sup>H} δ 32.5 (CH<sub>2</sub>—P—CH<sub>2</sub>, m, 1P); 35.4 (CH<sub>2</sub>—P—CH<sub>2</sub>, m, 1P).

**MS**: (–) 1614.5 [M–H<sup>+</sup>]<sup>–</sup>. (+) 1616.4 [M+H<sup>+</sup>]<sup>+</sup>.

## Example 27: Synthesis of compound 22



To a glass vial (4 mL),  $3 \cdot 3.5\text{H}_2\text{O}$  (35.6 mg; 79.6  $\mu\text{mol}$ ; 1.0 equiv.) was added. Methylene-bis(phosphonic acid) (27.6 mg; 192  $\mu\text{mol}$ ; 2.4 equiv.), paraformaldehyde (5.8 mg; 193  $\mu\text{mol}$ ; 2.4 equiv.) and aqueous HCl (6 M; 1 mL) were subsequently added and the resulting suspension was stirred overnight at 80 °C. The mixture was evaporated to dryness and the residue was several times co-evaporated with  $\text{H}_2\text{O}$ . The crude product was purified by ion exchange chromatography (DOWEX 50;  $\text{H}^+$ -form;  $\text{H}_2\text{O}$ ). Fractions with product were combined, evaporated to dryness and further co-evaporated with  $\text{H}_2\text{O}$ . Residue was re-dissolved in  $\text{H}_2\text{O}$  (50 mL) and subsequently lyophilized. Product was obtained as a white substance (21.2 mg; 47%; 1 step, based on  $3 \cdot 3.5\text{H}_2\text{O}$ ).

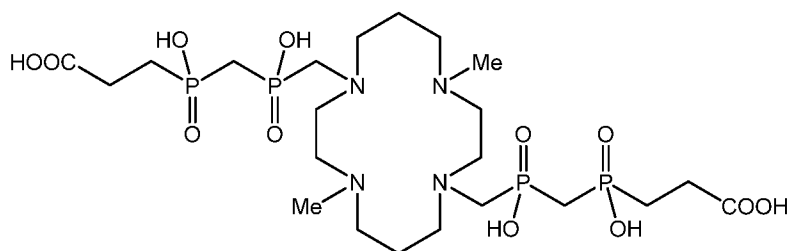
**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.71 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , p, 4H,  $^3J_{\text{HH}} = 7$  Hz); 2.04 (P— $\text{CH}_2$ —P, dd, 2H,  $^2J_{\text{HP}} = 18$  Hz,  $^2J_{\text{HP}} = 16$  Hz); 2.23 ( $\text{CH}_3$ , s, 6H); 2.51 (*cycle*, t, 4H,  $^3J_{\text{HH}} = 7$  Hz); 2.57 (*cycle*, m, 4H); 2.75 (*cycle*, m, 4H); 2.81 (N— $\text{CH}_2$ —P, d, 2H,  $^2J_{\text{HP}} = 9$  Hz); 2.86 (*cycle*, m, 4H); 7.13 (PH, d,  $^1J_{\text{HP}} = 531$  Hz);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  20.2 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , s); 35.7 (P— $\text{CH}_2$ —P, t,  $^1J_{\text{CP}} = 77$  Hz); 44.0 ( $\text{CH}_3$ , s); 48.7 (*cycle*, s); 49.9 (*cycle*, d,  $^3J_{\text{CP}} = 7$  Hz); 51.9 (*cycle*, d,  $^3J_{\text{CP}} = 7$  Hz); 53.8 (*cycle*, s); 57.0 (N— $\text{CH}_2$ —P, d,  $^1J_{\text{CP}} = 110$  Hz);  $^{31}\text{P}$   $\delta$  19.8 (PH, dtd, 2P,  $^1J_{\text{PH}} = 531$  Hz,  $^2J_{\text{PH}} = 18$  Hz,  $^2J_{\text{PP}} = 3$  Hz); 32.0 (N—CH—P, m, 2P).

**MS**: (–) 539.3 [ $\text{M—H}^+$ ] $^-$ . (+) 541.4 [ $\text{M+H}^+$ ] $^+$ ; 563.4 [ $\text{M+Na}^+$ ] $^+$ ; 585.4 [ $\text{M—H}^++2\text{Na}^+$ ] $^+$ ; 607.4 [ $\text{M—2H}^++3\text{Na}^+$ ] $^+$ .

**TLC** ( $\text{SiO}_2$ , *i*-PrOH–conc.  $\text{NH}_4\text{OH—H}_2\text{O}$  7:3:3):  $R_f = 0.7$ .

**EA** ( $\text{C}_{16}\text{H}_{40}\text{N}_4\text{O}_8\text{P}_4 \cdot 1.5\text{H}_2\text{O}$ ,  $M_R = 567.4$ ): C 33.9 (33.8); H 7.6 (7.2); N 9.9 (9.6).

## Example 28: Synthesis of compound 23



To a glass vial (20 ml), 1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane tetrahydrochloride (295 mg; 788  $\mu\text{mol}$ ; 1.0 equiv.) was added. Intermediate **D** (792 mg; 3,66 mmol; 4,6 equiv.), paraformaldehyde (49.7 mg; 1.66 mmol; 2.1 equiv.) and aqueous HCl (6 M; 15 mL) were subsequently added and the resulting suspension was stirred at 80 °C for three days. Mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The crude product was purified by column chromatography (SiO<sub>2</sub>; EtOH–conc. aq. NH<sub>4</sub>OH 1:1;  $R_f$  = 0.5). Fractions with product were joined and evaporated to dryness. Residue was re-dissolved in H<sub>2</sub>O (25 mL), treated with charcoal and resulting suspension was filtered through syringe microfilter (Millipore; 0.22  $\mu\text{m}$ ). Filtrate was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were combined and evaporated to dryness. The residue was re-dissolved in H<sub>2</sub>O (500 mL) and subsequently lyophilized. Product was obtained as a white substance (392 mg; 62 %; 1 step, based on 1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane tetrahydrochloride).

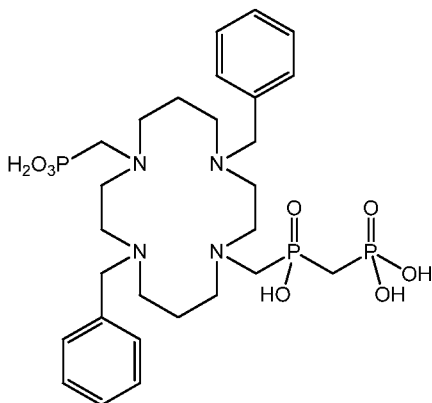
**NMR** (D<sub>2</sub>O): <sup>1</sup>H  $\delta$  1.80 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 4H, <sup>3</sup>J<sub>HH</sub> = 6 Hz); 1.95 (P—CH<sub>2</sub>—C, m, 4H); 2.10 (P—CH<sub>2</sub>—P, dd, 4H, <sup>2</sup>J<sub>HP</sub> = 17 Hz, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.25 (CH<sub>3</sub>, s, 6H); 2.35 (CH<sub>2</sub>—CO, tm, 4H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); 2.49 (cycle, t, 4H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.51 (cycle, m, 2H); 2.58 (cycle, m, 4H); 2.63–2.79 (cycle, N—CH<sub>2</sub>—P, m, 10H); <sup>13</sup>C{<sup>1</sup>H}  $\delta$  23.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 29.2 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 97 Hz); 30.8 (CH<sub>2</sub>—CO, s); 31.0 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 79; <sup>1</sup>J<sub>CP</sub> = 76 Hz); 43.8 (CH<sub>3</sub>, s); 50.2 (cycle, d, <sup>3</sup>J<sub>CP</sub> = 6 Hz); 50.6 (cycle, s); 52.6 (cycle, s); 54.2 (cycle, s); 55.5 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 110 Hz); 184.1 (CO, d, <sup>3</sup>J<sub>CP</sub> = 17 Hz); <sup>31</sup>P{<sup>1</sup>H}  $\delta$  33.8 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 34.8 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz).

**MS**: (–) 665.2 [M–H<sub>3</sub>O<sup>+</sup>]<sup>–</sup>; 683.3 [M–H<sup>+</sup>]<sup>–</sup>. (+) 685.2 [M+H<sup>+</sup>]<sup>+</sup>; 707.2 [M+Na<sup>+</sup>]<sup>+</sup>; 729.2 [M–H<sup>+</sup>+2Na<sup>+</sup>]<sup>+</sup>; 751.1 [M–2H<sup>+</sup>+3Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. NH<sub>4</sub>OH 1:1):  $R_f$  = 0.5.

**EA** (C<sub>22</sub>H<sub>48</sub>N<sub>4</sub>O<sub>12</sub>P<sub>4</sub>·3H<sub>2</sub>O,  $M_R$  = 738.6): C 35.8 (35.5); H 7.6 (8.0); N 7.6 (7.7).

## Example 29: Synthesis of compound 24



To a glass vial (4 ml), **6**·3.5HCl·H<sub>2</sub>O (81.3 mg; 116 μmol; 1.0 equiv.) was added. Phosphorous acid (90.2 mg; 1.10 mmol; 9.5 equiv.), paraformaldehyde (8.2 mg; 273 μmol; 2.4 equiv.) and aqueous HCl (12 M; 1 mL) were subsequently added and the resulting suspension was stirred at 40 °C for five days. The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were combined and evaporated to dryness. The residue was several times co-evaporated with H<sub>2</sub>O and re-dissolved in H<sub>2</sub>O (50 mL). The resulting solution was subsequently lyophilized. Product was obtained as a white substance (40.4 mg; 51 %; 1 step, based on **6**·3.5HCl·H<sub>2</sub>O).

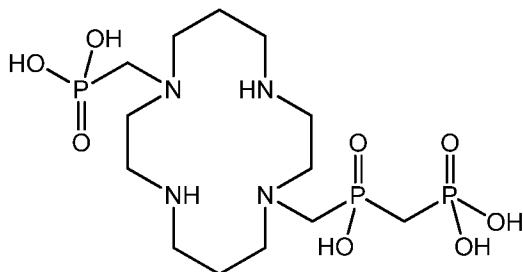
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.78–1.94 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 4H); 2.12 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 19 Hz, <sup>2</sup>J<sub>HP</sub> = 17 Hz); 2.51–2.60 (*cycle*, m, 2H); 2.63–2.81 (*cycle*, bm, 4H); 2.88–2.98 (*cycle*, N—CH<sub>2</sub>—P, m, 4H); 3.03–3.44 (*cycle*, N—CH<sub>2</sub>—P, m, 10H); 3.94 (CH<sub>2</sub>—Ph, bm, 2H); 4.02 (CH<sub>2</sub>—Ph, bm, 2H); 6.99–7.30 (*Ph*, m, 8H); <sup>13</sup>C {<sup>1</sup>H} δ 22.6 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 27.6 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 34.8 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 117 Hz, <sup>1</sup>J<sub>CP</sub> = 75 Hz); 41.5 (*cycle*, s); 42.4 (*cycle*, s); 50.4 (*cycle*, s); 51.3 (*cycle*, s); 52.3 (*cycle*, s); 52.9 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 104 Hz); 53.3 (*cycle*, s); 54.8 (*cycle*, s); 56.2 (CH<sub>2</sub>—Ph, s); 56.9 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 8 Hz); 58.9 (CH<sub>2</sub>—Ph, s); 59.0 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 110 Hz); 123.3 (*Ph*, s); 124.5 (*Ph*, s); 129.0 (*Ph*, s); 129.2 (*Ph*, s); 130.3 (*Ph*, s); 131.4 (*Ph*, s); 137.2 (*Ph*, s); 142.8 (*Ph*, s); <sup>31</sup>P {<sup>1</sup>H} 19.2 (HO—P—OH, m, 1P); 26.9 (HO—P—OH, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 34.5 (N—CH—P, bm, 1P).

**MS**: (–) 645.0 [M–H<sup>+</sup>]<sup>–</sup>. (+) 647.1 [M+H<sup>+</sup>]<sup>+</sup>; 669.2 [M+Na<sup>+</sup>]<sup>+</sup>; 685.2 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.8.

EA (C<sub>27</sub>H<sub>45</sub>N<sub>4</sub>O<sub>8</sub>P<sub>3</sub>·2H<sub>2</sub>O, M<sub>R</sub> = 682.6): C 47.5 (47.6); H 7.2 (7.2); N 8.2 (8.2).

Example 30: *Synthesis of compound 25*



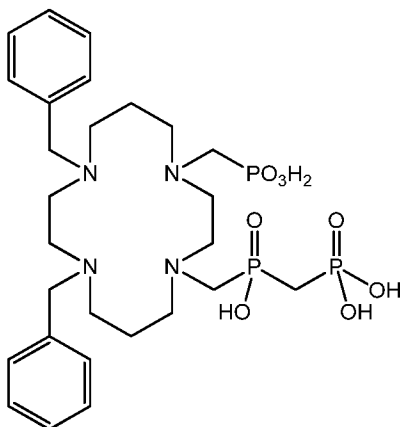
5 In a glass flask (50 ml), **24**·2H<sub>2</sub>O (26.3 mg; 38.5 μmol; 1.0 equiv.) was dissolved in H<sub>2</sub>O (25 mL) and the resulting mixture was briefly washed with gaseous argon. Ammonium formate (98.2 mg; 1.56 mmol; 41 equiv.) and Pd@C catalyst (10 %; 9.1 mg) were subsequently added. Resulting suspension was stirred at 60 °C overnight. The mixture was then evaporated to dryness and several times co-  
 10 evaporated with H<sub>2</sub>O. The residue was dissolved in H<sub>2</sub>O, the catalyst was filtered off and the filtrate was evaporated to dryness. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Aqueous fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was re-  
 15 dissolved in H<sub>2</sub>O (50 mL) and lyophilized. Product was obtained as a white substance (15.4 mg; 76%; 1 step, based on **24**·2H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.85–1.98 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 4H); 2.11 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 18 Hz, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.48–2.84 (*cycle*, bm, 6H); 2.88 (*cycle*, m, 2H); 3.01 (N—CH<sub>2</sub>—P, d, 2H, <sup>2</sup>J<sub>HP</sub> = 12 Hz); 3.08–3.38 (*cycle*, N—CH<sub>2</sub>—P, m, 10H); <sup>13</sup>C{<sup>1</sup>H} δ 23.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 27.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 34.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> =  
 20 117 Hz, <sup>1</sup>J<sub>CP</sub> = 76 Hz); 43.8 (*cycle*, s); 42.4 (*cycle*, s); 50.4 (*cycle*, s); 51.3 (*cycle*, s); 52.6 (*cycle*, s); 52.8 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 107 Hz); 54.1 (*cycle*, s); 55.2 (*cycle*, s); 57.6 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 111 Hz); 58.2 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 8 Hz); <sup>31</sup>P{<sup>1</sup>H} 28.3 (HO—P—OH, d, 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz); 32.6 (N—CH—P, d, 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz).

**MS**: (–) 465.2 [M–H<sup>+</sup>]<sup>–</sup>. (+) 467.3 [M+H<sup>+</sup>]<sup>+</sup>; 489.2 [M+Na<sup>+</sup>]<sup>+</sup>; 505.2 [M+K<sup>+</sup>]<sup>+</sup>; 511.2  
 25 [M–H<sup>+</sup>+2Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.4.

EA (C<sub>13</sub>H<sub>33</sub>N<sub>4</sub>O<sub>8</sub>P<sub>3</sub>·3.5H<sub>2</sub>O, M<sub>R</sub> = 529.4): C 29.5 (29.3); H 7.6 (7.9); N 10.6 (10.7).

Example 31: *Synthesis of compound 26*

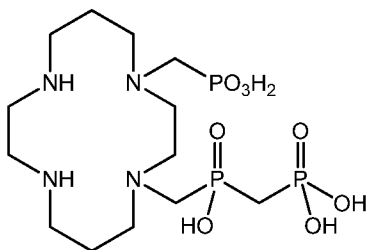
To a glass vial (4 ml), 7·4H<sub>2</sub>O (91.3 mg; 150 μmol; 1.0 equiv.) was added. Phosphorous acid (123 mg; 1.5 mmol; 10 equiv.), paraformaldehyde (10.8 mg; 360 μmol; 2.4 equiv.) and aqueous HCl (12 M; 1 mL) were subsequently added and the resulting suspension was stirred at 40 °C for five days. The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were joined and evaporated to dryness. The residue was several times co-evaporated with H<sub>2</sub>O and re-dissolved in H<sub>2</sub>O (50 mL). Resulting solution was subsequently lyophilized. Product was obtained as a white substance (59.4 mg; 58 %; 1 step, based on 7·4H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): 1.72–1.84 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 1.92 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.05–2.33 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, P—CH<sub>2</sub>—P bm, 3H); 2.49–3.24 (*cycle*, N—CH<sub>2</sub>—P, bm, 20H); 3.82 (CH<sub>2</sub>—Ph, m, 2H); 4.07 (CH<sub>2</sub>—Ph, m, 2H); 6.83–6.92 (*Ph*, bm, 3H); 6.98–7.22 (*Ph*, bm, 8H); <sup>31</sup>P{<sup>1</sup>H} 18.3 (HO—P—OH, s, 1P); 22.9 (HO—P—OH, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 32.4 (N—CH—P, bm, 1P).

**MS**: (–) 645.1 [M–H<sup>+</sup>]<sup>–</sup>. (+) 647.4 [M+H<sup>+</sup>]<sup>+</sup>; 669.5 [M+Na<sup>+</sup>]<sup>+</sup>; 685.4 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.8.

**EA** (C<sub>27</sub>H<sub>45</sub>N<sub>4</sub>O<sub>8</sub>P<sub>3</sub>·2H<sub>2</sub>O, M<sub>R</sub> = 682.6): C 47.2 (47.6); H 7.3 (7.2); N 8.0 (8.2).

Example 32: *Synthesis of compound 27*

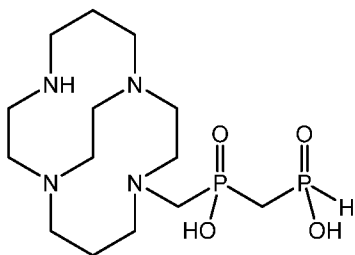
In a glass flask (50 ml), **26**·2H<sub>2</sub>O (28 mg; 41 μmol; 1.0 equiv.) was dissolved in H<sub>2</sub>O (25 mL) and the resulting mixture was briefly washed with gaseous argon. Ammonium formate (107.8 mg; 1.71 mmol; 45 equiv.) and Pd@C catalyst (10 %; 9.1 mg) were subsequently added. The resulting suspension was stirred at 60 °C overnight. The mixture was then evaporated to dryness and several times evaporated with H<sub>2</sub>O. The residue was dissolved in H<sub>2</sub>O, the catalyst was filtered off and the filtrate was evaporated to dryness. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Aqueous fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was re-dissolved in H<sub>2</sub>O (50 mL) and lyophilized. Product was obtained as a white substance (15.4 mg; 82%; 1 step, based on **26**·2H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): **NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.88 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 1.97–2.08 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 2.13–2.35 (P—CH<sub>2</sub>—P, CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 3H); 2.44–3.38 (cycle, N—CH<sub>2</sub>—P, m, 20H); <sup>13</sup>C{<sup>1</sup>H} δ 22.8 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 25.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.1 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 114 Hz, <sup>1</sup>J<sub>CP</sub> = 80 Hz); 42.1 (cycle, s); 45.5 (cycle, s); 49.9 (cycle, s); 51.1 (cycle, s); 51.5 (cycle, s); 53.9 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); 54.6 (cycle, s); 54.8 (cycle, s); 57.6 (cycle, s); 59.2 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 108 Hz); <sup>31</sup>P{<sup>1</sup>H} 21.3 (HO—P—OH, m, 1P); 28.3 (HO—P—OH, d, 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz); 32.2 (N—CH—P, d, 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz).

**MS**: (–) 465.4 [M–H<sup>+</sup>]<sup>–</sup>. (+) 467.2 [M+H<sup>+</sup>]<sup>+</sup>; 489.5 [M+Na<sup>+</sup>]<sup>+</sup>; 505.5 [M+K<sup>+</sup>]<sup>+</sup>; 511.6 [M–H<sup>+</sup>+2Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.4.

**EA** (C<sub>13</sub>H<sub>33</sub>N<sub>4</sub>O<sub>8</sub>P<sub>3</sub>·5H<sub>2</sub>O, M<sub>R</sub> = 556.4): C 28.3 (28.1); H 8.6 (8.8); N 9.8 (10.1).

Example 33: *Synthesis of compound 28*

To a glass vial (20 ml), 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (1.17 g; 5.16 mmol; 2.4 equiv.) was added. Methylene-bis(phosphinic acid) (633 mg; 4.40 mmol; 2.0 equiv.), paraformaldehyde (65.3 mg; 2.17 mmol; 1.0 equiv.) and aqueous HCl (5 M; 20 mL) were subsequently added and the resulting suspension was stirred 80 °C overnight. The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (Amberlite IRA 402; OH<sup>-</sup>-form; H<sub>2</sub>O → 10 % aqueous AcOH). Acetate fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was dissolved in aqueous HCl (5 M; 10 mL) and the resulting solution was evaporated to dryness. The residue was further dried on vacuum pump and then in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. The final product was obtained as a white substance (668 mg; 63%; 1 step; based on paraformaldehyde).

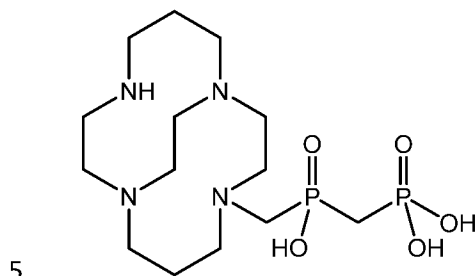
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.70 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, dm, 1H, <sup>1</sup>J<sub>HH</sub> = 17 Hz); 1.76 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, dm, 1H, <sup>1</sup>J<sub>HH</sub> = 17 Hz); 2.12 (P—CH<sub>2</sub>—P, m, 2H); 2.30 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.30 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.56 (*cycle*, m, 2H); 2.63 (*cycle*, m, 2H); 2.92 (*cycle*, m, 3H); 3.05 (*cycle*, m, 2H); 3.14 (*cycle*, m, 2H); 3.17 (N—CH<sub>2</sub>—P, 1H); 3.22 (*cycle*, m, 1H); 3.30 (*cycle*, m, 3H); 3.44 (*cycle*, m, 2H); 3.62 (*cycle*, m, 1H); 3.73 (N—CH<sub>2</sub>—P, m, 1H); 3.78 (*cycle*, m, 2H); 7.13 (PH, d, 1H, <sup>1</sup>J<sub>HP</sub> = 533 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 18.9 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 20.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 36.0 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 84 Hz; <sup>1</sup>J<sub>CP</sub> = 77 Hz); 42.2 (*cycle*, s); 47.7 (*cycle*, s); 49.5 (*cycle*, s); 49.9 (*cycle*, s); 51.7 (CH<sub>2</sub>—N—CH<sub>2</sub>—P, d, <sup>3</sup>J<sub>CP</sub> = 6 Hz); 54.0 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 91 Hz); 54.4 (*cycle*, s); 56.2 (*cycle*, s); 58.2 (*cycle*, s); 58.4 (*cycle*, s); 59.5 (*cycle*, s); <sup>31</sup>P δ 20.6 (PH, dtd, 1P, <sup>1</sup>J<sub>PH</sub> = 533 Hz, <sup>2</sup>J<sub>PH</sub> = 17 Hz, <sup>2</sup>J<sub>PP</sub> = 6 Hz); 25.7 (P—CH<sub>2</sub>—N, m, 1P).

**MS:** (–) 381.2 [M–H<sup>+</sup>]<sup>–</sup>; 402.9 [M–2H<sup>+</sup>+Na<sup>+</sup>]<sup>–</sup>. (+) 383.3 [M+H<sup>+</sup>]<sup>+</sup>; 405.3 [M+Na<sup>+</sup>]<sup>+</sup>; 421.2 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.7.

EA ( $C_{14}H_{32}N_4O_4P_2 \cdot 2.5HCl \cdot H_2O$ ,  $M_R = 491.5$ ): C 34.2 (34.4); H 7.5 (7.7); N 11.4 (11.0).

Example 34: *Synthesis of compound 29*



To a glass vial (50 ml), 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (954 mg; 4.22 mmol; 2.0 equiv.) was added. Intermediate **A**·0.5H<sub>2</sub>O (360 mg; 2.13 mmol; 1.0 equiv.), paraformaldehyde (81.9 mg; 2.73 mmol; 1.3 equiv.) and aqueous HCl (5 M; 20 mL) were subsequently added and the resulting suspension was stirred 80 °C overnight. The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (Amberlite IRA 402; OH<sup>-</sup>-form; H<sub>2</sub>O → 10 % aqueous AcOH). Acetate fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was dissolved in aqueous HCl (6 M; 10 mL) and the resulting solution was evaporated to dryness. The residue was further dried on vacuum pump and in later in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. The final product was obtained as a white substance (571 mg; 58%; 1 step; based on **A**·0.5H<sub>2</sub>O).

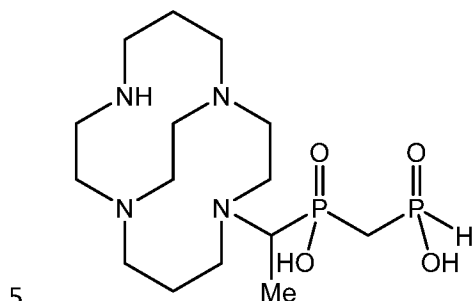
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.66 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, dm, 1H, <sup>1</sup>J<sub>HH</sub> = 17 Hz); 1.75 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, dm, 1H, <sup>1</sup>J<sub>HH</sub> = 17 Hz); 1.92 (P—CH<sub>2</sub>—P, m, 2H); 2.20 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.33 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.60 (*cycle*, m, 2H); 2.71 (*cycle*, m, 2H); 2.91 (*cycle*, m, 2H); 3.01 (*cycle*, m, 6H); 3.03 (N—CH<sub>2</sub>—P, 1H); 3.15 (*cycle*, m, 1H); 3.29 (*cycle*, m, 4H); 3.79 (*cycle*, m, 3H); 4.24 (N—CH<sub>2</sub>—P, m, 1H); <sup>13</sup>C{<sup>1</sup>H} δ 19.5 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 20.6 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.7 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 115 Hz; <sup>1</sup>J<sub>CP</sub> = 88 Hz); 42.3 (*cycle*, s); 48.8 (*cycle*, s); 50.0 (*cycle*, s); 50.1 (*cycle*, s); 52.3 (CH<sub>2</sub>—N—CH<sub>2</sub>—P, d, <sup>3</sup>J<sub>CP</sub> = 7 Hz); 53.2 (*cycle*, s); 53.4 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 87 Hz); 55.0 (*cycle*, s); 58.7 (*cycle*, s); 59.1 (*cycle*, s); 59.4 (*cycle*, s); <sup>31</sup>P{<sup>1</sup>H} δ 11.3 (HO—P—OH, m, 1P); 27.6 (P—CH<sub>2</sub>—N, m, 1P).

**MS**: (+) 399.1 [M+H<sup>+</sup>]<sup>+</sup>; 421.0 [M+Na<sup>+</sup>]<sup>+</sup>; 437.2 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.2.

EA ( $C_{14}H_{32}N_4O_5P_2 \cdot 1.5HCl \cdot 0.5H_2O$ ,  $M_R = 462.1$ ): C 36.4 (36.2); H 7.5 (7.5); N 12.1 (12.0).

Example 35: *Synthesis of compound 30*



To a glass vial (4 mL), 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (298 mg; 1.32 mmol; 4.5 equiv.) was added. Methylene-bis(phosphinic acid) (93.5 mg; 649  $\mu$ mol; 2.2 equiv.), acetaldehyde (13.0 mg; 295  $\mu$ mol; 1.0 equiv.) and aqueous HCl (6 M; 20 mL) were subsequently added and the resulting suspension was stirred 60 °C overnight. The mixture was then evaporated to dryness and several times co-evaporated with  $H_2O$ . The residue was purified by ion exchange chromatography (DOWEX 50;  $H^+$ -form;  $H_2O \rightarrow 10\%$  aqueous pyridine). Pyridine fraction with product was evaporated to dryness and several times co-evaporated with  $H_2O$ . The residue was re-dissolved in  $H_2O$  (50 mL) and subsequently lyophilized. The final product was obtained as a white substance (62.3 mg; 51%; 1 step; based on acetaldehyde).

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**NMR** ( $D_2O$ ):  $^1H$   $\delta$  1.19 ( $CH_3$ , dd, 3H,  $^3J_{HP} = 15$  Hz,  $^2J_{HH} = 6$  Hz); 1.72 ( $CH_2-CH_2-CH_2$ , dm, 1H,  $^1J_{HH} = 16$  Hz); 1.80 ( $CH_2-CH_2-CH_2$ , dm, 1H,  $^1J_{HH} = 17$  Hz); 2.15 ( $P-CH_2-P$ , t, 2H,  $^2J_{HP} = 16$  Hz); 2.27–2.39 ( $CH_2-CH_2-CH_2$ , m, 2H); 2.48 (*cycle*, m, 2H); 2.68 (*cycle*, m, 2H); 2.95–3.04 (*cycle*, m, 4H); 3.21–3.37 (*cycle*, bm, 11H); 3.51 ( $CH-CH_3$ , dq, 1H,  $^2J_{HP} = 14$  Hz,  $^2J_{HH} = 6$  Hz); 3.62 (*cycle*, m, 2H); 3.78 (*cycle*, m, 1H); 7.15 (*PH*, d, 1H,  $^1J_{HP} = 533$  Hz);  $^{13}C\{^1H\}$  8.9 ( $CH_3$ , s);  $\delta$  22.1 ( $CH_2-CH_2-CH_2$ , s); 23.5 ( $CH_2-CH_2-CH_2$ , s); 36.7 ( $P-CH_2-P$ , dd,  $^1J_{CP} = 81$  Hz;  $^1J_{CP} = 75$  Hz); 41.6 (*cycle*, s); 46.2 (*cycle*, s); 49.7 (*cycle*, s); 50.9 (*cycle*, d,  $^3J_{CP} = 6$  Hz); 51.2 (*cycle*, s); 53.5 ( $N-CH_2-P$ , d,  $^1J_{CP} = 92$  Hz); 54.1 (*cycle*, s); 55.5 (*cycle*, s); 56.8 (*cycle*, s); 57.2 (*cycle*, s); 59.9 (*cycle*, s);  $^{31}P$   $\delta$  19.8 (*PH*, dtm, 1P,  $^1J_{PH} = 533$  Hz,  $^2J_{PH} = 16$  Hz); 29.2 ( $P-CH_2-N$ , m, 1P).

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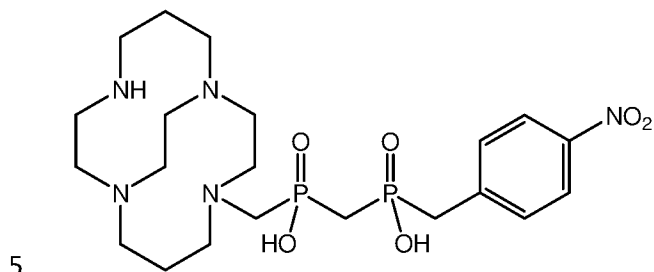
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**MS**: (–) 394.9 [ $M-H^+$ ] $^-$ . (+) 396.9 [ $M+H^+$ ] $^+$ ; 419.0 [ $M+Na^+$ ] $^+$ ; 441.0 [ $M-H^++2Na^+$ ] $^+$ .

TLC (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 2:1): *R<sub>f</sub>* = 0.7.

EA (C<sub>15</sub>H<sub>34</sub>N<sub>4</sub>O<sub>4</sub>P<sub>2</sub>·H<sub>2</sub>O, *M<sub>R</sub>* = 414.2): C 43.5 (43.8); H 8.8 (8.5); N 13.5 (13.5).

Example 36: *Synthesis of compound 31*



To a glass vial (20 mL), 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (1.12 g; 4.97 μmol; 1.6 equiv.) was added. Intermediate **B** (856 mg; 3.07 mmol; 1.0 equiv.), paraformaldehyde (92.4 mg; 3.08 mmol; 1.0 equiv.) and aqueous HCl (6 M; 15 mL) were subsequently added and the resulting suspension was stirred 80 °C overnight.

10 The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10% aqueous pyridine). Pyridine fraction with product was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The crude product was purified by flash chromatography (C18; gradient elution H<sub>2</sub>O—MeCN). Fractions with pure

15 product were combined and evaporated to dryness. The residue was re-dissolved in H<sub>2</sub>O (250 mL) and subsequently lyophilized. The final product was obtained as a white substance (1.04 g; 55 %; 1 step; based on intermediate **B**).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.69 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 1.77 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.10 (P—CH<sub>2</sub>—P, m, 2H); 2.27 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.39 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.59 (*cycle*, m, 2H); 2.64 (*cycle*, m, 2H); 2.85–3.07 (*cycle*, m, 5H); 3.07–3.35 (*cycle*, P—CH<sub>2</sub>—C, N—CH<sub>2</sub>—P, m, 9H); 3.38 (*cycle*, m, 1H); 3.38 (*cycle*, m, 1H); 3.68 (*cycle*, m, 1H); 3.74–3.88 (*cycle*, N—CH<sub>2</sub>—P, m, 3H); 7.51 (CH—C—CH<sub>2</sub>, dd, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz, <sup>4</sup>J<sub>HP</sub> = 2 Hz); 8.19 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz); <sup>13</sup>C {<sup>1</sup>H} δ 19.0 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 20.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.3 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 85 Hz, <sup>1</sup>J<sub>CP</sub> = 82 Hz); 41.1 (P—CH<sub>2</sub>—C, dd, <sup>1</sup>J<sub>CP</sub> = 86 Hz, <sup>3</sup>J<sub>CP</sub> = 3 Hz); 42.2 (*cycle*, s); 47.9 (*cycle*, s); 49.6 (*cycle*, s); 49.9 (*cycle*, s); 51.8 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 7 Hz); 54.1 (*cycle*, s); 55.2 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 90 Hz); 58.4 (*cycle*, s); 58.7 (*cycle*, s); 59.5 (*cycle*, s); 124.4 (CH—C—N, s); 131.3 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz); 143.9 (CH—C—CH<sub>2</sub>, d,

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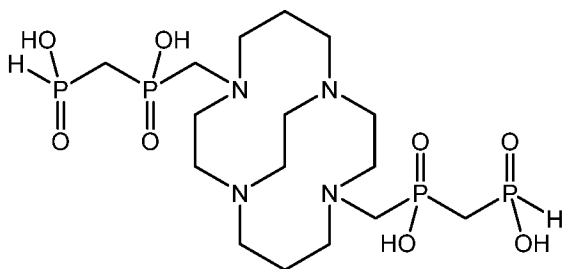
$^2J_{CP} = 8$  Hz); 146.8 (CH—C—N, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  23.3 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P,  $^2J_{PP} = 11$  Hz); 31.1 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P,  $^2J_{PP} = 11$  Hz).

MS: (-) 516.2 [M—H<sup>+</sup>]<sup>-</sup>.

TLC (SiO<sub>2</sub>, EtOH—conc. aq. NH<sub>4</sub>OH 4:1):  $R_f = 0.6$ .

5 EA (C<sub>21</sub>H<sub>37</sub>N<sub>5</sub>O<sub>6</sub>P<sub>2</sub>·5.5H<sub>2</sub>O,  $M_R = 616.5$ ): C 40.9 (40.8); H 7.9 (7.6); N 11.4 (11.7).

Example 37: *Synthesis of compound 32*



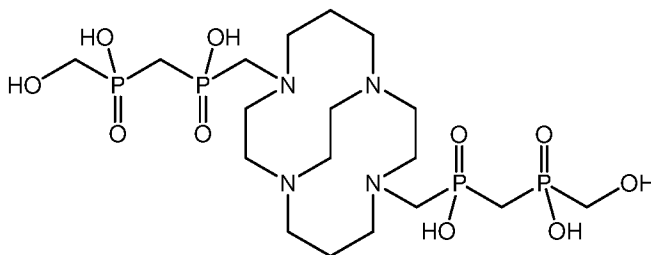
To a glass flask (25 mL), 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (214 mg; 945  $\mu\text{mol}$ ; 1.0 equiv.) was added. Methylene-bis(phosphonic acid) (835 mg; 5.80 mmol; 5.5 equiv.), paraformaldehyde (63.5 mg; 2.12 mmol; 2.0 equiv.) and aqueous HCl (6 M; 5 mL) were subsequently added and the resulting suspension was stirred at 80 °C for two days. The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were joined and evaporated to dryness. The residue was re-dissolved in H<sub>2</sub>O (200 mL) and subsequently lyophilized. The final product was obtained as a white substance (392 mg; 68 %; 1 step; based on 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane).

20 NMR (D<sub>2</sub>O):  $^1\text{H}$   $\delta$  1.67 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 1.82 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 1.99 (P—CH<sub>2</sub>—P, t, 4H,  $^2J_{HP} = 17$  Hz); 2.78–3.19 (N—CH<sub>2</sub>, bm, 20H); 3.26 (N—CH<sub>2</sub>, bm, 2H); 3.62 (N—CH<sub>2</sub>, bm, 2H); 7.14 (PH, d, 2H,  $^1J_{HP} = 530$  Hz);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  24.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 37.1 (P—CH<sub>2</sub>—P, dd,  $^1J_{CP} = 76$  Hz,  $^1J_{CP} = 72$  Hz); 52.4 (*cycle*, s); 52.8 (*cycle*, s); 53.8 (CH<sub>2</sub>—N—CH<sub>2</sub>—P, d,  $^3J_{CP} = 4$  Hz); 53.8 (N—CH<sub>2</sub>—P, d,  $^1J_{CP} = 100$  Hz); 56.5 (*cycle*, s); 57.1 (*cycle*, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  19.8 (PH, dt, 2P,  $^1J_{PH} = 530$  Hz,  $^2J_{PH} = 18$  Hz); 32.5 (P—CH<sub>2</sub>—N, t, 2P,  $^2J_{PH} = 15$  Hz).

MS: (-) 536.7 [M—H<sup>+</sup>]<sup>-</sup>.

TLC (SiO<sub>2</sub>, *i*PrOH—conc. aq. NH<sub>4</sub>OH—H<sub>2</sub>O 7:3:3):  $R_f = 0.4$ .

EA (C<sub>16</sub>H<sub>38</sub>N<sub>4</sub>O<sub>8</sub>P<sub>4</sub>·4H<sub>2</sub>O,  $M_R = 610.5$ ): C 31.5 (31.3); H 7.6 (7.4); N 9.2 (9.2).

Example 38: *Synthesis of compound 33*

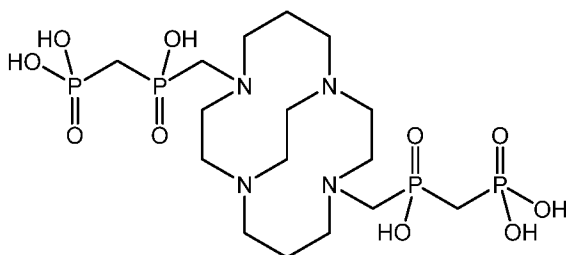
To a glass vial (4 mL), **32**·4H<sub>2</sub>O (59.8 mg; 98 μmol; 1.0 equiv.) was added and it was followed by addition of paraformaldehyde (13.6 mg; 453 μmol; 4.6 equiv.) and aqueous HCl (6 M; 10 mL). The resulting suspension was stirred at 80 °C overnight. On the next day, the mixture was evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The crude product was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were combined and evaporated to dryness. The residue was re-dissolved in H<sub>2</sub>O (25 mL) and subsequently lyophilized. The final product was obtained as a white substance (51.3 mg; 80 %; 1 step; based on **32**·4H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.82 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.06 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.30 (P—CH<sub>2</sub>—P, t, 4H, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.67–3.63 (*cycle*, N—CH<sub>2</sub>—P, bm, 24H); 3.67 (O—CH<sub>2</sub>—P, d, 4H, <sup>2</sup>J<sub>HP</sub> = 5 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 22.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 34.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 84 Hz, <sup>1</sup>J<sub>CP</sub> = 73 Hz); 51.4 (*cycle*, s); 52.8 (*cycle*, s); 53.6 (CH<sub>2</sub>—N—CH<sub>2</sub>—P, d, <sup>3</sup>J<sub>CP</sub> = 4 Hz); 54.9 (*cycle*, s); 55.2 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 98 Hz); 57.8 (*cycle*, s); 61.3 (P—CH<sub>2</sub>—O, d, <sup>3</sup>J<sub>CP</sub> = 108 Hz) <sup>31</sup>P{<sup>1</sup>H} δ 27.6 (P—CH<sub>2</sub>—N, bm, 2P); 40.6 (P—CH<sub>2</sub>—O, m, 2P).

**MS**: (–) 596.8 [M–H<sup>+</sup>]<sup>–</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.5.

**EA** (C<sub>18</sub>H<sub>42</sub>N<sub>4</sub>O<sub>10</sub>P<sub>4</sub>·3H<sub>2</sub>O, M<sub>R</sub> = 652.5): C 33.1 (33.4); H 7.4 (7.5); N 8.6 (8.3).

Example 39: *Synthesis of compound 34*

To a glass flask (20 mL),  $32 \cdot 4\text{H}_2\text{O}$  (91.6 mg; 150  $\mu\text{mol}$ ; 1.0 equiv.) was added. Solution of  $\text{HgCl}_2$  (173 mg; 638  $\mu\text{mol}$ ; 4.3 equiv.) in aqueous HCl (2 M; 5 mL) was added and the mixture was stirred at 60 °C for two days. Precipitate was filtered off and the mother liquor was saturated with  $\text{H}_2\text{S}$ . Precipitate was centrifuged off, washed  
 5 with  $\text{H}_2\text{O}$  and the supernatant was evaporated to dryness. The residue was re-dissolved in aqueous HCl (3 %; 300  $\mu\text{L}$ ) followed by addition of excess of *i*PrOH (25 mL). On the next day, mother liquor was decanted off and the oily residue was several times co-evaporated with  $\text{H}_2\text{O}$ . The residue was re-dissolved in  $\text{H}_2\text{O}$  (100 mL) and subsequently lyophilized. Product was obtained as a white substance (68.4 mg; 68  
 10 %; 1 step, based on  $32 \cdot 4\text{H}_2\text{O}$ ).

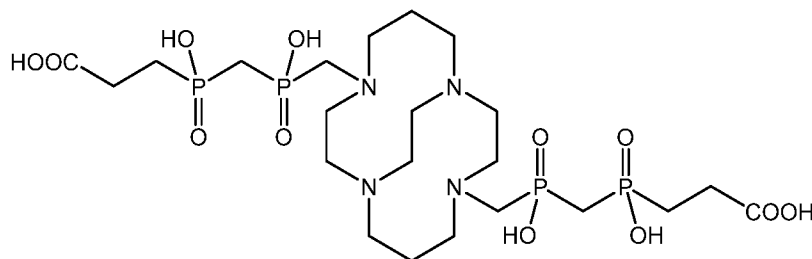
**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.68 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , bm, 2H); 1.81 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , bm, 2H); 1.92 (P— $\text{CH}_2$ —P, t, 4H,  $^2J_{\text{HP}} = 18$  Hz); 2.75–3.21 (N— $\text{CH}_2$ , bm, 20H); 3.27 (N— $\text{CH}_2$ , bm, 2H); 3.66 (N— $\text{CH}_2$ , bm, 2H);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  24.5 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , s); 35.1 (P— $\text{CH}_2$ —P, dd,  $^1J_{\text{CP}} = 117$  Hz,  $^1J_{\text{CP}} = 75$  Hz); 52.5 (*cycle*, s); 52.9 (*cycle*, s);  
 15 53.3 (N— $\text{CH}_2$ —P, bm); 53.7 ( $\text{CH}_2\text{—N—CH}_2\text{—P}$ , bm); 56.7 (*cycle*, s); 57.0 (*cycle*, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  12.4 (HO—P—OH, d, 2P,  $^2J_{\text{PP}} = 7$  Hz); 36.7 (P— $\text{CH}_2$ —N, d, 2P,  $^2J_{\text{PP}} = 7$  Hz).

**TLC** ( $\text{SiO}_2$ , *i*PrOH–conc. aq.  $\text{NH}_4\text{OH—H}_2\text{O}$  7:3:3):  $R_f = 0.1$ .

**MS**: (–) 569.2 [ $\text{M—H}^+$ ] $^-$ . (+) 571.4 [ $\text{M+H}^+$ ] $^+$ ; 593.4 [ $\text{M+Na}^+$ ] $^+$ ; 609.4 [ $\text{M+K}^+$ ] $^+$ .

20 **EA** ( $\text{C}_{16}\text{H}_{38}\text{N}_4\text{O}_{10}\text{P}_4 \cdot 2.5\text{HCl} \cdot \text{H}_2\text{O}$ ,  $M_R = 679,6$ ): C 28.3 (28.4); H 6.3 (6.2); N 8.2 (8.3).

Example 40: *Synthesis of compound 35*



To a glass vial (20 mL), 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (148 mg; 653  $\mu\text{mol}$ ; 1.0 equiv.) was added. Intermediate **D** (688 mg; 3.18 mmol; 4.9 equiv.), paraformaldehyde (48.6 mg; 1.62 mmol; 2.5 equiv.) and aqueous HCl (12 M; 10 mL) were subsequently added and the resulting suspension was stirred at 80 °C for two days. The mixture was then evaporated to dryness and several times co-evaporated with  $\text{H}_2\text{O}$ . The residue was purified by column chromatography ( $\text{SiO}_2$ ; EtOH–conc.  
 25

aq.  $\text{NH}_4\text{OH}$  1:1;  $R_f = 0.4$ ). Fractions with product were combined and evaporated to dryness. The residue was re-dissolved in  $\text{H}_2\text{O}$  (25 mL), treated with small amounts of charcoal and filtered through syringe microfilter (Millipore; 0.22  $\mu\text{m}$ ). The filtrate was further purified by ion exchange chromatography (DOWEX 50;  $\text{H}^+$ -form;  $\text{H}_2\text{O}$ ).

5 Fractions with product were combined and evaporated to dryness. The residue was re-dissolved in  $\text{H}_2\text{O}$  (125 mL) and subsequently lyophilized. The final product was obtained as a white substance (272 mg; 58 %; 1 step; based on 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane).

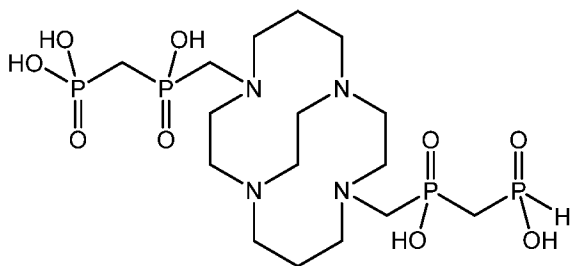
10 **NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.72 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , m, 2H); 1.74 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , m, 2H); 1.94 (P— $\text{CH}_2$ —P, P— $\text{CH}_2$ —C, m, 8H); 2.48 ( $\text{CH}_2\text{—CO}$ , t, 4H,  $^3J_{\text{HH}} = 7$  Hz); 2.92–3.31 (*cycle*, N— $\text{CH}_2$ , bm, 22H); 3.52 (N— $\text{CH}_2$ , bm, 2H);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  24.4 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , s); 26.8 (P— $\text{CH}_2$ —C, d,  $^1J_{\text{CP}} = 94$  Hz); 31.0 ( $\text{CH}_2\text{—CO}$ , s); 34.9 (P— $\text{CH}_2$ —P, dd,  $^1J_{\text{CP}} = 76$  Hz,  $^1J_{\text{CP}} = 74$  Hz); 52.4 (*cycle*, s); 52.8 (*cycle*, s); 54.2 (N— $\text{CH}_2$ —P, d,  $^1J_{\text{CP}} = 102$  Hz); 54.4 (*cyclus*, d,  $^3J_{\text{CP}} = 5$  Hz); 57.1 (*cycle*, s); 57.3 (*cycle*, s); 182.8 (CO, d,  $^3J_{\text{CP}} = 15$  Hz);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  30.4 ( $\text{CH}_2\text{—P—CH}_2$ , d, 2P,  $^2J_{\text{PP}} = 10$  Hz); 33.7 ( $\text{CH}_2\text{—P—CH}_2$ , d, 2P,  $^2J_{\text{PP}} = 10$  Hz).

**MS**: (–) 663.3 [ $\text{M—H}_3\text{O}^+$ ] $^-$ ; 681.3 [ $\text{M—H}^+$ ] $^-$ . (+) 683.2 [ $\text{M+H}^+$ ] $^+$ ; 727.2 [ $\text{M—H}^++2\text{Na}^+$ ] $^+$ .

**TLC** ( $\text{SiO}_2$ , EtOH–conc.  $\text{NH}_4\text{OH}$  1:1):  $R_f = 0.3$ .

20 **EA** ( $\text{C}_{22}\text{H}_{46}\text{N}_4\text{O}_{12}\text{P}_4 \cdot 2\text{H}_2\text{O}$ ,  $M_R = 718.6$ ): C 36.8 (36.9); H 7.0 (7.3); N 7.8 (7.5).

Example 41: *Synthesis of compound 36*



25 To a glass vial (4 mL),  $\mathbf{29} \cdot 1.5\text{HCl} \cdot 0.5\text{H}_2\text{O}$  (51.3 mg; 111  $\mu\text{mol}$ ; 1.0 equiv.) was added. Methylene-bis(phosphinic acid) (59.2 mg; 411  $\mu\text{mol}$ ; 3.7 equiv.), paraformaldehyde (4.9 mg; 163  $\mu\text{mol}$ ; 1.5 equiv.) and aqueous HCl (12 M; 1 mL) were subsequently added and the resulting suspension was stirred at 80  $^\circ\text{C}$  for two days. The mixture was then evaporated to dryness and several times co-evaporated with  $\text{H}_2\text{O}$ . The residue was purified by ion exchange chromatography (DOWEX 50;

H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were combined and evaporated to dryness. The residue was dried in vacuum pump and subsequently in vacuum desiccator over P<sub>2</sub>O<sub>5</sub>. Resulting product was obtained as a white substance (35.0 mg; 55 %; 1 step; based on 29·1.5HCl·0.5H<sub>2</sub>O).

5 **NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.83–2.11 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 4H); 2.24 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.37 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 18 Hz, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.64–3.44 (cycle, N—CH<sub>2</sub>—P, bm, 24H); <sup>13</sup>C{<sup>1</sup>H} δ 22.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 23.7 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 34.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 122 Hz, <sup>1</sup>J<sub>CP</sub> = 82 Hz); 36.3 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 82 Hz); 49.3–57.4 (12 × N—CH<sub>2</sub>, bm); <sup>31</sup>P δ 19.0 (HO—P—OH, m, 1P);  
10 24.3 (PH, dm, 1P, <sup>1</sup>J<sub>PH</sub> = 533 Hz); 26.7 (P—CH<sub>2</sub>—N, bm, 1P); 27.8 (P—CH<sub>2</sub>—N, bm, 1P).

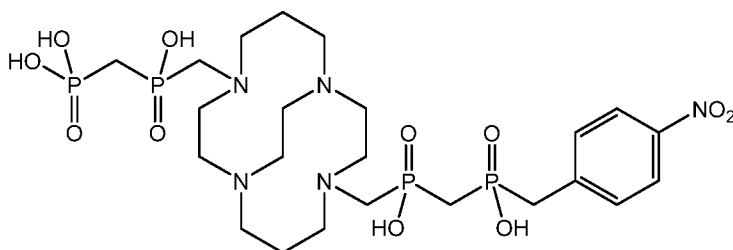
**MS**: (–) 553.1 [M–H<sup>+</sup>]<sup>–</sup>. (+) 555.2 [M+H<sup>+</sup>]<sup>+</sup>; 577.2 [M+Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.3.

**EA** (C<sub>16</sub>H<sub>38</sub>N<sub>4</sub>O<sub>9</sub>P<sub>4</sub>·H<sub>2</sub>O, M<sub>R</sub> = 572.4): C 33.6 (33.4); H 7.0 (6.7); N 9.8 (9.9).

15

Example 42: *Synthesis of compound 37*



To a glass vial (4 mL), 29·1.5HCl·0.5H<sub>2</sub>O (403 mg; 872 μmol; 1.0 equiv.) was added. Intermediate **B** (294 mg; 1.05 mmol; 1.2 equiv.), paraformaldehyde (39.3 mg;  
20 1.33 mmol; 1.5 equiv.) and aqueous HCl (12 M; 5 mL) were subsequently added and the resulting suspension was stirred at 80 °C for three days. The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by flash chromatography (C18; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN). Fractions with pure product were combined and evaporated to dryness.  
25 The residue was re-dissolved in H<sub>2</sub>O (250 mL) and subsequently lyophilized. Resulting product was obtained as a yellow substance (417 mg; 62 %; 1 step; based on 29·1.5HCl·0.5H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 2.02 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 2.32 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 2.40 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.48 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 20 Hz,

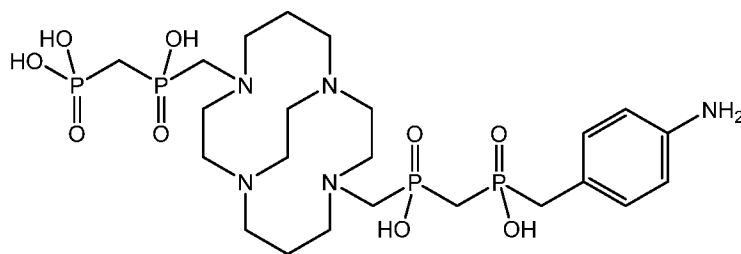
$^2J_{HP} = 17$  Hz); 2.79–3.98 (*cycle*, N—CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, bm, 26H); 7.57 (CH, dd, 2H,  $^3J_{HH} = 9$  Hz,  $^4J_{HP} = 2$  Hz); 8.24 (CH, d, 2H,  $^3J_{HH} = 9$  Hz);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  20.5 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 21.0 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 32.2 (P—CH<sub>2</sub>—P, dd,  $^1J_{CP} = 124$  Hz,  $^1J_{CP} = 84$  Hz); 33.2 (P—CH<sub>2</sub>—P, t,  $^1J_{CP} = 82$  Hz); 39.3 (P—CH<sub>2</sub>—C, d,  $^1J_{CP} = 89$  Hz); 48.0–59.6 (12 × N—CH<sub>2</sub>, bm); 124.5 (CH—C—N, d,  $^4J_{CP} = 3$  Hz); 131.5 (CH—C—CH<sub>2</sub>, d,  $^3J_{CP} = 6$  Hz); 141.8 (CH—C—CH<sub>2</sub>, d,  $^2J_{CP} = 9$  Hz); 147.2 (CH—C—N, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  18.0 (HO—P—OH, m, 1P); 24.0 (P—CH<sub>2</sub>—N, bm, 1P); 25.6 (P—CH<sub>2</sub>—N, bm, 1P); 39.0 (P—CH<sub>2</sub>—C, m, 1P).

**MS:** (–) 687.8 [M–H<sup>+</sup>]<sup>–</sup>; 709.7 [M–2H<sup>+</sup>+Na<sup>+</sup>]<sup>–</sup>; 725.7 [M–2H<sup>+</sup>+K<sup>+</sup>]<sup>–</sup>. (+) 689.9 [M+H<sup>+</sup>]<sup>+</sup>; 711.9 [M+Na<sup>+</sup>]<sup>+</sup>; 727.8 [M+K<sup>+</sup>]<sup>+</sup>; 749.8 [M–H<sup>+</sup>+Na<sup>+</sup>+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): *R*<sub>f</sub> = 0.3.

**EA** (C<sub>23</sub>H<sub>43</sub>N<sub>5</sub>O<sub>11</sub>P<sub>4</sub>·4.5H<sub>2</sub>O, *M*<sub>R</sub> = 770.6): C 28.3 (28.4); H 6.3 (6.2); N 8.2 (8.3).

Example 43: *Synthesis of compound 38*



In a glass flask (250 ml), **37**·4.5H<sub>2</sub>O (288 mg; 374  $\mu$ mol) was dissolved in H<sub>2</sub>O (100 mL) and the resulting mixture was briefly washed with gaseous argon. Pd@C catalyst (10 %; 44 mg) was subsequently added and the resulting suspension was then treated with gaseous H<sub>2</sub> at 60 °C overnight. The catalyst was then filtered off and the filtrate was evaporated to dryness. The residue was re-dissolved in H<sub>2</sub>O (250 mL) and subsequently lyophilized. Resulting product was obtained as a yellowish substance (257 mg; 94 %; 1 step; based on **37**·4.5H<sub>2</sub>O).

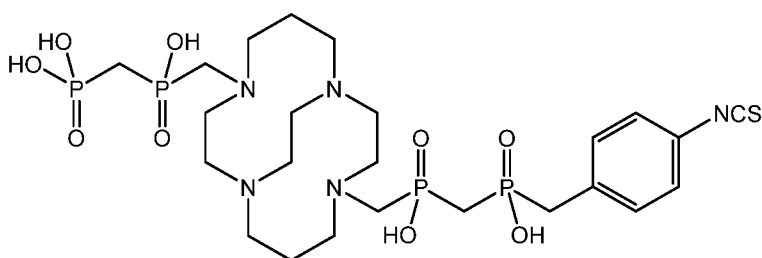
**NMR** (D<sub>2</sub>O):  $^1\text{H}$   $\delta$  1.80 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 1.86 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 2.10 (P—CH<sub>2</sub>—P, m, 2H); 2.21 (P—CH<sub>2</sub>—P, m, 2H); 2.65–3.77 (*cycle*, N—CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, bm, 26H); 6.97 (CH, d, 2H,  $^3J_{HH} = 8$  Hz); 7.25 (CH, d, 2H,  $^3J_{HH} = 8$  Hz);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  20.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 20.5 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 32.7 (P—CH<sub>2</sub>—P, m); 33.4 (P—CH<sub>2</sub>—P, m); 33.6 (P—CH<sub>2</sub>—C, d,  $^1J_{CP} = 91$  Hz); 46.7–57.2 (12 × N—CH<sub>2</sub>, bm); 123.0 (CH—C—N, s); 118.2 (CH—C—N, m); 130.8 (CH—C—CH<sub>2</sub>,

d,  $^3J_{CP} = 5$  Hz); 140.3 (CH—C—CH<sub>2</sub>, m);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  13.5 (HO—P—OH, bm, 1P); 24.6 (P—CH<sub>2</sub>—N, bm, 1P); 23.6 (P—CH<sub>2</sub>—N, bm, 1P); 32.6 (P—CH<sub>2</sub>—C, bm, 1P).  
**MS:** (-) 658.0 [M—H<sup>+</sup>]<sup>-</sup>; 679.9 [M—2H<sup>+</sup>+Na<sup>+</sup>]<sup>-</sup>. (+) 659.9 [M+H<sup>+</sup>]<sup>+</sup>; 981.9 [M+Na<sup>+</sup>]<sup>+</sup>; 697.8 [M+K<sup>+</sup>]<sup>+</sup>.

5 **TLC** (SiO<sub>2</sub>, *i*PrOH—conc. aq. NH<sub>4</sub>OH—H<sub>2</sub>O 7:3:3):  $R_f = 0.3$ .

**EA** (C<sub>23</sub>H<sub>45</sub>N<sub>5</sub>O<sub>9</sub>P<sub>4</sub>·4H<sub>2</sub>O,  $M_R = 731.6$ ): C 37.8 (37.6); H 7.3 (7.2); N 9.6 (9.5).

Example 44: *Synthesis of compound 39*

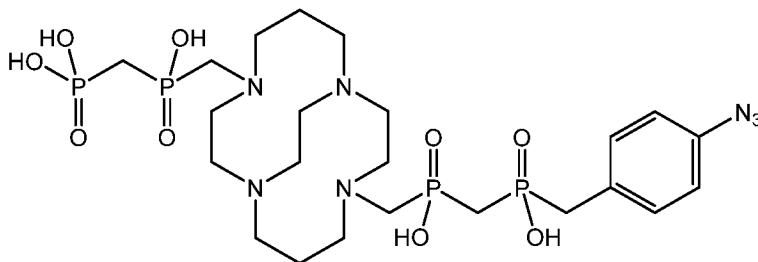


10 In a glass flask (100 mL), **38**·4H<sub>2</sub>O (48.3 mg; 66,0  $\mu\text{mol}$ , 1.0 equiv.) was dissolved in H<sub>2</sub>O (20 mL). Freshly prepared solution of CSCI<sub>2</sub> (15 mM; 20 mL; 300  $\mu\text{mol}$ ; 4.5 equiv.) in CCl<sub>4</sub> was then added and the resulting biphasic mixture was vigorously stirred at room temperature overnight. The organic layer was separated off and the aqueous layer was evaporated to dryness. The crude product was purified by  
 15 preparative HPLC (C8; gradient elution H<sub>2</sub>O—0.1% TFA—MeCN). Fraction with product was directly lyophilized. Product was obtained as a white substance (35.2 mg; 67 %; 1 step; based on **38**·4H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O):  $^1\text{H}$   $\delta$  2.02 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 2.29 (P—CH<sub>2</sub>—P, t, 2H,  $^2J_{HP} = 16$  Hz); 2.32 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm, 2H); 2.37 (P—CH<sub>2</sub>—P, t, 2H,  $^2J_{HP} = 19$  Hz);  
 20 2.86–3.92 (*cycle*, N—CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, bm, 26H); 7.37 (CH, d, 2H,  $^3J_{HH} = 8$  Hz); 7.39 (CH, m, 2H);  $^{13}\text{C}\{^1\text{H}\}$  20.8 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 21.3 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 31.7 (P—CH<sub>2</sub>—P, dd,  $^1J_{CP} = 120$  Hz,  $^1J_{CP} = 83$  Hz); 32.3 (P—CH<sub>2</sub>—P, t,  $^1J_{CP} = 82$  Hz); 38.4 (P—CH<sub>2</sub>—C, d,  $^1J_{CP} = 91$  Hz); 46.6–59.1 (12  $\times$  N—CH<sub>2</sub>, bm); 126.7 (CH—C—N, s); 130.1 (CH—C—N, s); 131.8 (CH—C—CH<sub>2</sub>, s); 132.2 (CH—C—CH<sub>2</sub>, d,  
 25  $^2J_{CP} = 8$  Hz); 134.8 (CS, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  16.3 (HO—P—OH, m, 1P); 23.4 (P—CH<sub>2</sub>—N, bm, 1P); 23.6 (P—CH<sub>2</sub>—N, bm, 1P); 38.3 (P—CH<sub>2</sub>—C, m, 1P).

**MS:** (-) 699.6 [M—H<sup>+</sup>]<sup>-</sup>; 721.6 [M—2H<sup>+</sup>+Na<sup>+</sup>]<sup>-</sup>; 737.6 [M—2H<sup>+</sup>+K<sup>+</sup>]<sup>-</sup>. (+) 701.8 [M+H<sup>+</sup>]<sup>+</sup>; 723.8 [M+Na<sup>+</sup>]<sup>+</sup>; 739.8 [M+K<sup>+</sup>]<sup>+</sup>.

**EA** (C<sub>24</sub>H<sub>43</sub>N<sub>5</sub>O<sub>9</sub>P<sub>4</sub>S·3.5H<sub>2</sub>O,  $M_R = 796.1$ ): C 36.2 (36.2); H 6.3 (6.3); N 8.8 (8.7).

Example 45: *Synthesis of compound 40*

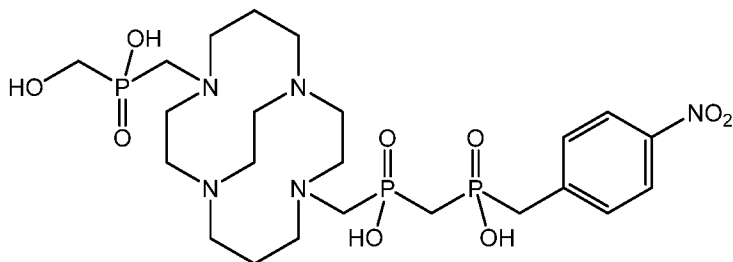
In a glass vial (4 mL), **37**·4H<sub>2</sub>O (161 mg; 220 μmol; 1.0 equiv.) was dissolved  
 5 in aqueous HCl (243.8 mM; 1800 μL; 440 μmol; 2.0 equiv.) and the resulting mixture  
 was cooled in an ice bath (1 °C). Freshly prepared aqueous solution of NaNO<sub>2</sub> was then  
 gradually added (290 mM; 1.0 mL; 290 μmol; 1.3 equiv.; 100 μL each three minutes).  
 Freshly prepared aqueous solution of NaN<sub>3</sub> (580 mM; 760 μL; 441 μmol; 2.0 equiv.)  
 10 was then added. The reaction mixture was then stirred at room temperature for three  
 hours. The crude product was divided into two halves which were successively  
 purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN).  
 Fraction with product were combined and directly lyophilized. Product was obtained  
 as a white substance (29.5 mg; 57 %; 1 step; based on **37**·4H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 2.07 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.18 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m);  
 15 2.28–2.41 (P—CH<sub>2</sub>—P, m, 4H); 2.70–3.82 (*cycle*, N—CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, bm,  
 26H); 7.17 (CH—C—N, d, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 7.31 (CH—C—CH<sub>2</sub>, dd, 2H, <sup>3</sup>J<sub>HH</sub> = 7  
 Hz, <sup>H</sup>J<sub>HP</sub> = 2 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 20.5 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 22.5 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s);  
 32.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 121 Hz, <sup>1</sup>J<sub>CP</sub> = 82 Hz); 32.3 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 82  
 Hz); 36.6 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 93 Hz); 47.2–60.1 (12 × N—CH<sub>2</sub>, bm); 123.5 (CH—  
 20 C—N, s); 131.7 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 4 Hz); 132.2 (CH—C—CH<sub>2</sub>, d, <sup>2</sup>J<sub>CP</sub> = 7  
 Hz); 141.0 (CH—C—N, s); <sup>31</sup>P{<sup>1</sup>H} δ 17.5 (HO—P—OH, m, 1P); 22.2 (P—CH<sub>2</sub>—N,  
 bm, 1P); 24.0 (P—CH<sub>2</sub>—N, bm, 1P); 36.8 (P—CH<sub>2</sub>—C, m, 1P).

**MS**: (–) 666.0 [M–H<sub>3</sub>O<sup>+</sup>]<sup>–</sup>; 684.1 [M–H<sup>+</sup>]<sup>–</sup>. (+) 688.1 [M+H<sup>+</sup>]<sup>+</sup>; 708.1 [M+Na<sup>+</sup>]<sup>+</sup>;  
 724.0 [M+K<sup>+</sup>]<sup>+</sup>; 730.1 [M–H<sup>+</sup>+2Na<sup>+</sup>]<sup>+</sup>.

25 **TLC** (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.2.

**EA** (C<sub>23</sub>H<sub>43</sub>N<sub>7</sub>O<sub>9</sub>P<sub>4</sub>·3H<sub>2</sub>O, M<sub>R</sub> = 739.6): C 37.4 (37.2); H 6.7 (6.6); N 13.3 (13.3).

Example 46: Synthesis of compound **41**

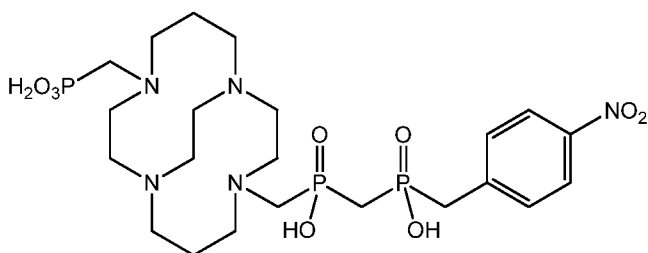
To a glass vial (20 mL), **31**·5.5H<sub>2</sub>O (50.9 mg; 82.6 μmol; 1.0 equiv.) was added. Paraformaldehyde (10.1 mg; 343 μmol; 4.2 equiv.), and hydroxymethylphosphinic acid (50.8 mg; 529 μmol; 6.4 equiv.), and aqueous HCl (6 M; 2 mL) were subsequently added and the resulting suspension was stirred at 80 °C overnight. The mixture was then evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The residue was purified by ion exchange chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). Fractions with product were combined, evaporated to dryness and several times co-evaporated with H<sub>2</sub>O. The crude product was purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0,1% TFA–MeCN). Fraction with product was directly lyophilized. Resulting product was obtained as a yellowish substance (25.8 mg; 46 %; 1 step; based on **31**·5.5H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.84 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 2.15–2.55 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, P—CH<sub>2</sub>—P, m, 4H); 2.76 (*cycle*, m, 4H); 2.98 (*cycle*, m, 2H); 3.09 (*cycle*, m, 2H); 3.18–3.84 (*cycle*, N—CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, O—CH<sub>2</sub>—P, m, 20H); 7.57 (CH—C—CH<sub>2</sub>, d, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); 8.25 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 20.7 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.5 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 81 Hz); 39.5 (P—CH<sub>2</sub>—C, dd, <sup>1</sup>J<sub>CP</sub> = 88 Hz); 49.1 (*cycle*, s); 49.2 (*cycle*, s); 50.2 (*cycle*, s); 51.7 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 84 Hz); 54.6 (*cycle*, s); 54.9 (*cycle*, s); 58.3 (*cycle*, s); 58.9 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 83 Hz); 58.9 (P—CH<sub>2</sub>—O, d, <sup>1</sup>J<sub>CP</sub> = 116 Hz); 124.6 (CH—C—N, s); 131.6 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz); 142.3 (CH—C—CH<sub>2</sub>, d, <sup>2</sup>J<sub>CP</sub> = 8 Hz); 147.2 (CH—C—N, s); <sup>31</sup>P{<sup>1</sup>H} δ 23.6 (P—CH<sub>2</sub>—P, bm, 1P); 29.7 (P—CH<sub>2</sub>—P, bm, 1P); 37.3 (P—CH<sub>2</sub>—O, m, 1P).

**MS**: (–) 623.5 [M–H<sup>+</sup>]<sup>–</sup>. (+) 625.4 [M+H<sup>+</sup>]<sup>+</sup>; 647.7 [M+Na<sup>+</sup>]<sup>+</sup>; 663.5 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 2:1): R<sub>f</sub> = 0.6.

**EA** (C<sub>23</sub>H<sub>42</sub>N<sub>5</sub>O<sub>9</sub>P<sub>3</sub>·3H<sub>2</sub>O, M<sub>R</sub> = 679.6): C 40.7 (40.6); H 7.1 (7.1); N 10.3 (10.5).

Example 47: *Synthesis of compound 42*

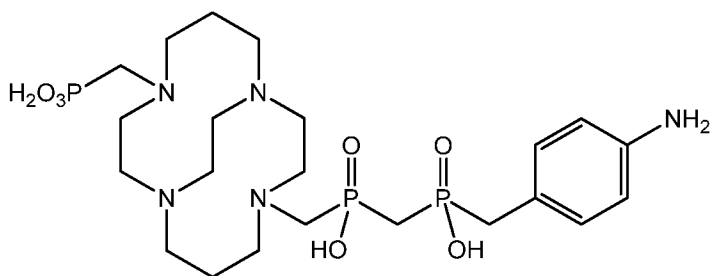
To a glass vial (20 mL),  $31 \cdot 5.5\text{H}_2\text{O}$  (336 mg; 545  $\mu\text{mol}$ ; 1.0 equiv.) was added. Paraformaldehyde (44.2 mg; 1.47 mmol; 2.7 equiv.), and phosphorous acid (380 mg; 4.63 mmol; 8.5 equiv.), and aqueous HCl (12 M; 10 mL) were subsequently added and the resulting suspension was stirred at 40 °C for four days. The mixture was then evaporated to dryness and several times co-evaporated with  $\text{H}_2\text{O}$ . The residue was purified by ion exchange chromatography (DOWEX 50;  $\text{H}^+$ -form;  $\text{H}_2\text{O}$ ). Fractions with product were combined, evaporated to dryness and several times co-evaporated with  $\text{H}_2\text{O}$ . The crude product was purified by flash chromatography (C18; gradient elution  $\text{H}_2\text{O}$ –0.1% TFA–MeCN). Fraction with product was directly lyophilized. Resulting product was obtained as a yellow substance (194 mg; 54 %; 1 step; based on  $31 \cdot 5.5\text{H}_2\text{O}$ ).

**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.85 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , m, 2H); 2.12 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , m, 1H); 2.27 (P— $\text{CH}_2$ —P, m, 4H); 2.35 ( $\text{CH}_2\text{—CH}_2\text{—CH}_2$ , m, 1H); 2.66–2.72 (*cycle*, m, 3H); 2.89 (*cycle*, m, 2H); 2.98–3.12 (*cycle*, m, 3H); 3.14–3.60 (*cycle*, N— $\text{CH}_2$ —P, P— $\text{CH}_2$ —C, m, 18H); 7.42 ( $\text{CH—C—CH}_2$ , d, 2H,  $^2J_{\text{HH}} = 8$  Hz); 8.20 ( $\text{CH—C—N}$ , d, 2H,  $^2J_{\text{HH}} = 8$  Hz);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  21.2 (HO—P—OH, s, 1P); 23.6 (P— $\text{CH}_2$ —P, d, 1P,  $^2J_{\text{PP}} = 8$  Hz); 29.7 (P— $\text{CH}_2$ —P, d, 1P,  $^2J_{\text{PP}} = 8$  Hz).

**MS:** (–) 609.9 [ $\text{M—H}^+$ ] $^-$ . (+) 612.1 [ $\text{M+H}^+$ ] $^+$ ; 634.2 [ $\text{M+Na}^+$ ] $^+$ ; 650.2 [ $\text{M+K}^+$ ] $^+$ .

**TLC** ( $\text{SiO}_2$ , EtOH–conc. aq.  $\text{NH}_4\text{OH}$  2:1):  $R_f = 0.4$ .

**EA** ( $\text{C}_{22}\text{H}_{40}\text{N}_5\text{O}_9\text{P}_3 \cdot 2.5\text{H}_2\text{O}$ ,  $M_r = 656.5$ ): C 40.3 (40.5); H 6.9 (7.0); N 10.7 (10.4).

Example 48: *Synthesis of compound 43*

In a glass flask (100 mL), **42**·2.5H<sub>2</sub>O (185 mg; 0.28 mmol; 1.0 equiv.) was dissolved in H<sub>2</sub>O (10 ml) and the flask was briefly washed with gaseous argon. Catalyst (10 % Pd@C; 54 mg) was then added and the resulting suspension was saturated with gaseous H<sub>2</sub> at 60 °C overnight. The catalyst was then filtered off and the filtrate was evaporated to dryness. The residue was re-dissolved in H<sub>2</sub>O (200 mL) and subsequently lyophilized. Resulting product was obtained as a white substance (158 mg; 89 %; 1 step; based on **42**·2.5H<sub>2</sub>O).

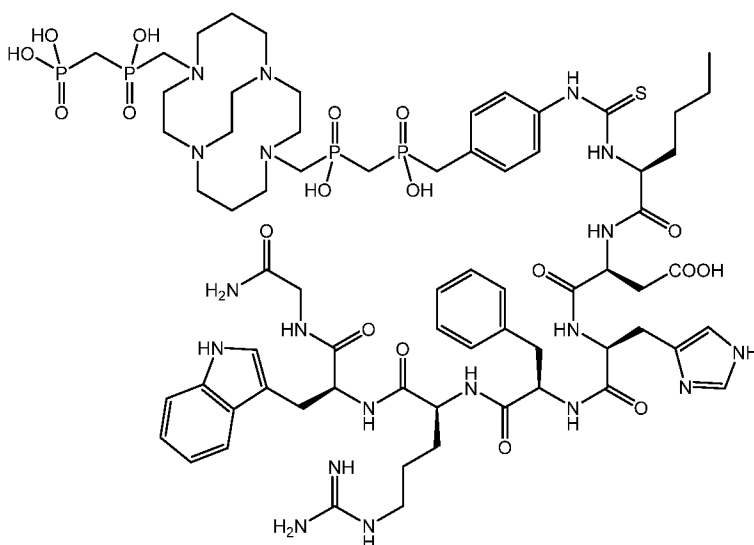
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.77 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 1.87 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.12 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.22–2.40 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, P—CH<sub>2</sub>—P, bm, 5H); 2.62–2.79 (*cycle*, m, 2H); 2.91–3.02 (*cycle*, m, 4H); 3.07–3.48 (*cycle*, N—CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, m, 20H); 6.89 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); 7.33 (CH—C—CH<sub>2</sub>, d, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 18.9 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 22.3 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 34.1 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 83 Hz); 37.5 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 90 Hz); 41.2–59.5 (12 × N—CH<sub>2</sub>, bm); 120.1 (CH—C—N, s); 127.1 (CH—C—N, m); 129.3 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz); 134.5 (CH—C—CH<sub>2</sub>, m); <sup>31</sup>P{<sup>1</sup>H} δ 19.3 (HO—P—OH, m, 1P); 22.4 (P—CH<sub>2</sub>—P, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 31.5 (P—CH<sub>2</sub>—P, bm, 1P).

**MS**: (–) 580.2 [M–H<sup>+</sup>]<sup>–</sup>. (+) 582.8 [M+H<sup>+</sup>]<sup>+</sup>; 603.2 [M+Na<sup>+</sup>]<sup>+</sup>; 619.8 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 2:1): R<sub>f</sub> = 0.3.

**EA** (C<sub>22</sub>H<sub>42</sub>N<sub>5</sub>O<sub>7</sub>P<sub>3</sub>·3H<sub>2</sub>O, M<sub>R</sub> = 635.6): C 41.2 (41.6); H 6.8 (6.6); N 10.7 (11.0).

Example 49: *Synthesis of targeting conjugate 44*



To a glass vial (4 mL), **39**·3.5H<sub>2</sub>O (5.3 mg; 6.7 μmol; 1.1 equiv.) was added. Peptide HNleAspHisD-PheArgTrpGlyNH<sub>2</sub> (5.9 mg; 6.4 μmol; 1.0 equiv.), aqueous

buffer  $\text{H}_3\text{BO}_3\text{-LiOH}$  (750 mM v  $\text{D}_2\text{O}$ ; pD = 10.1; 400  $\mu\text{L}$ ; 300  $\mu\text{mol}$ ; 50 equiv.) and  $\text{H}_2\text{O}$  (600  $\mu\text{L}$ ) were then added and the resulting mixture was stirred at room temperature overnight. The crude product was purified by preparative HPLC (C8; gradient elution  $\text{H}_2\text{O}$ -0.1% TFA-MeCN). Fraction with product was lyophilized.

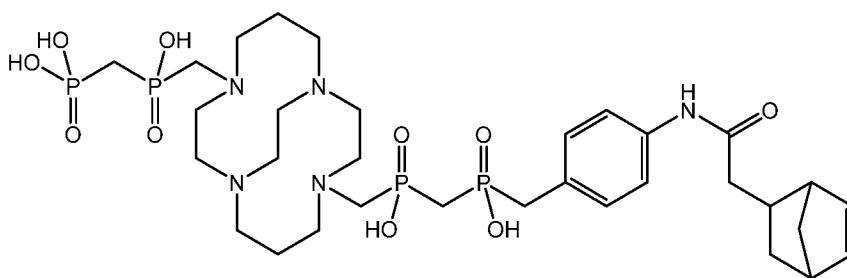
5 Resulting product was obtained as a white substance (5.7 mg; 55 %; 1 step; based on  $39 \cdot 3.5\text{H}_2\text{O}$ ).

NMR ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  0.86 ( $\text{CH}_3$ , t, 3H,  $^3J_{\text{HH}} = 7$  Hz); 1.01–1.53 ( $\text{CH}_2$ , bm, 10H); 1.68–2.29 ( $\text{CH}_2$ , P- $\text{CH}_2$ -P, *cycle*, bm, 8 H); 2.47–3.73 (*cycle*,  $\text{CH}_2$ , P- $\text{CH}_2$ -N, P- $\text{CH}_2$ -C, bm, 36H); 3.80 ( $\text{CH}_2$ , m, 2H); 4.11 ( $\text{CH}$ , m, 1H); 4.45 ( $\text{CH}$ , m, 1H); 4.51  
10 ( $\text{CH}$ , m, 1H); 4.55 ( $\text{CH}$ , m, 1H); 4.59 ( $\text{CH}$ , m, 1H); 4.69 ( $\text{CH}$ , m, 1H); 6.90 ( $\text{CH}_2$ -C- $\text{CH}$ -N, s, 1H); 7.14 ( $\text{CH}$ , t, 1H,  $^3J_{\text{HH}} = 7$  Hz); 7.14–7.27 (*arom.*, m, 6H); 7.29–7.38 (*arom.*, m, 5H); 7.46 ( $\text{CH}$ , d, 1H,  $^3J_{\text{HH}} = 8$  Hz); 7.66 ( $\text{CH}$ , d, 1H,  $^3J_{\text{HH}} = 8$  Hz); 8.03 (N- $\text{CH}$ -N, s, 1H);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  13.2 (HO-P-OH, bm, 1P); 25.3 (P- $\text{CH}_2$ -N, bm, 1P); 27.4 (P- $\text{CH}_2$ -N, bm, 1P); 32.3 (P- $\text{CH}_2$ -C, bm, 1P).

15 MS: (-) 1628.2  $[\text{M}-\text{H}^+]^-$ . (+) 1630.4  $[\text{M}+\text{H}^+]^+$ .

TLC ( $\text{SiO}_2$ , EtOH-conc. aq.  $\text{NH}_4\text{OH}$  1:1):  $R_f = 0.2$ .

#### Example 50: Synthesis of conjugate 45



20 In a glass vial (4 mL),  $38 \cdot 4\text{H}_2\text{O}$  (5.3 mg; 6.7  $\mu\text{mol}$ ; 1.1 equiv.) and *N*-hydroxysuccinimide ester of 5-norbornen-2-acetic acid (21.1 mg; 84.6  $\mu\text{mol}$ ; 2.2 equiv.) were dissolved in a mixture of aqueous buffer  $\text{H}_3\text{PO}_4\text{-NaOH}$  (1.0 M pH = 8.1; 1.00 mL; 1.0 mmol; 26 equiv.) and MeCN (0.5 mL). The resulting mixture was stirred at room temperature overnight. The crude product was purified by preparative HPLC (C8; gradient elution  $\text{H}_2\text{O}$ -0.1% TFA-MeCN). Fraction with product was lyophilized.  
25 Resulting product was obtained as a white substance (26.1 mg; 71 %; 1 step; based on  $38 \cdot 4\text{H}_2\text{O}$ ).

NMR ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.41–1.56 ( $\text{CH}-\text{CH}_2-\text{CH}$ ,  $\text{CH}_2$  bm, 2H); 1.62–1.75 ( $\text{CH}_2$ ,  $\text{CH}-\text{CH}_2-\text{CH}$ , bm, 2H); 1.94–2.32 (P- $\text{CH}_2$ -P, *cycle*, bm, 6H); 2.44 (P- $\text{CH}_2$ -P, t, 2H,

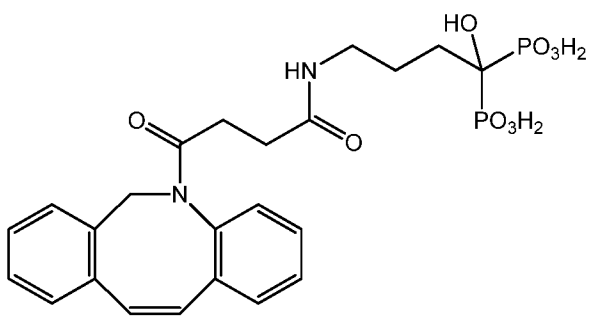
$^2J_{HP} = 17$  Hz); 2.84–4.16 (*cycle*, CH, N—CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, bm, 31H); 5.96 (CH, dd,  $^3J_{HH} = 12$  Hz,  $^3J_{HH} = 5$  Hz); 6.32 (CH, dd,  $^3J_{HH} = 12$  Hz,  $^3J_{HH} = 6$  Hz); 6.98 (CH, d, 2H,  $^3J_{HH} = 8$  Hz); 7.63 (CH, d, 2H,  $^3J_{HH} = 8$  Hz);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  21.1 (HO—P—OH, bm, 1P); 23.5 (P—CH<sub>2</sub>—N, bm, 1P); 24.9 (P—CH<sub>2</sub>—N, bm, 1P); 29.3 (P—CH<sub>2</sub>—C, bm, 1P).

MS: (–) 792.5 [M–H]<sup>+</sup>.

TLC (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.3.

EA (C<sub>32</sub>H<sub>55</sub>N<sub>5</sub>O<sub>10</sub>P<sub>4</sub>·TFA·2H<sub>2</sub>O, M<sub>R</sub> = 943.3): C 43.3 (43.4); H 6.4 (6.2); N 7.4 (7.7).

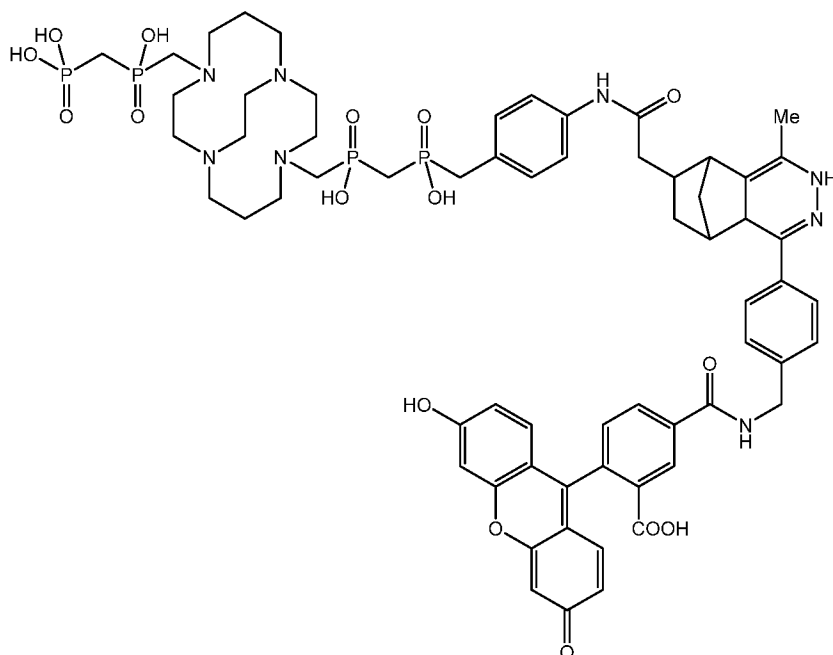
10 Example 51: *Synthesis of intermediate product F*



In a glass vial (4 mL), sodium aldendronate trihydrate (21.0 mg; 64.6  $\mu\text{mol}$ ; 1.4 equiv.) was dissolved in aqueous NaOH (250.6 mM; 773  $\mu\text{L}$ ; 194  $\mu\text{mol}$ ; 4.2 equiv.) and the resulting mixture was cooled in an ice bath (5 °C). Solution of *N*-hydroxysuccinimidester of 4-dibenzocyclooctynamidobutanoic acid (18.4 mg; 45.7  $\mu\text{mol}$ ; 1.0 equiv.) in dry DMSO (1.25 mL) was then added dropwise and the resulting mixture was stirred in the absence of light overnight. On the next day, the mixture was purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN). Fraction with product was lyophilized. Resulting product was obtained as a white substance (16.3 mg; 66 %; 1 step; based on *N*-hydroxysuccinimidester of 4-dibenzocyclooctynamidobutanoic acid).

NMR (D<sub>2</sub>O):  $^1\text{H}$   $\delta$  1.42 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 1.77 (CH<sub>2</sub>, m, 1H); 1.85 (CH<sub>2</sub>—C—P, tm,  $^3J_{HP} = 13$  Hz); 1.96 (CH<sub>2</sub>, t, 2H,  $^3J_{HH} = 7$  Hz); 2.16 (CH<sub>2</sub>, m, 1H); 2.88 (CH<sub>2</sub>—NH, t,  $^3J_{HH} = 9$  Hz); 3.61 (CH<sub>2</sub>—N—CO, d, 1H,  $^2J_{HH} = 14$  Hz); 5.04 (CH<sub>2</sub>—N—CO, d, 1H,  $^2J_{HH} = 14$  Hz); 7.29 (CH, d, 1H,  $^3J_{HH} = 7$  Hz); 7.35 (CH, t, 1H,  $^3J_{HH} = 7$  Hz); 7.39 (CH, t, 1H,  $^3J_{HH} = 7$  Hz); 7.42–7.53 (CH, m, 3H); 7.58 (CH, d, 1H,  $^3J_{HH} = 7$  Hz); 7.63 (CH, t, 1H,  $^3J_{HH} = 7$  Hz);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  21.2 (m, 1P).



Example 53: Synthesis of targeting conjugate **47**

In a glass vial (4 mL), fluorescein-6-methyl-tetrazine conjugate (1.0 mg; 1.8  $\mu\text{mol}$ ; 1.0 equiv.) was dissolved in dry DMSO (500  $\mu\text{L}$ ) followed by addition of freshly prepared solution of **45**·TFA·2H<sub>2</sub>O (2.5 mg; 2.7  $\mu\text{mol}$ ; 1.0 equiv.) in aqueous buffer AcOH–NH<sub>3</sub> (100 mM; 500  $\mu\text{L}$ ; 50  $\mu\text{mol}$ ; 28 equiv.). The resulting mixture was stirred at room temperature overnight. The crude product was purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN). Fraction with product was lyophilized. Resulting product (mixture of two regioisomers) was obtained as a white substance ( $\approx 2$  mg).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H  $\delta$  1.46 (CH–CH<sub>2</sub>–CH, m, 1H); 1.62 (CH<sub>2</sub>, bm, 1H); 1.88–1.96 (cycle, CH–CH<sub>2</sub>–CH, bm, 2H); 1.98–2.16 (P–CH<sub>2</sub>–P, cycle, bm, 3H); 2.25 (P–CH<sub>2</sub>–P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.32 (CH<sub>3</sub>, s, 3H); 2.63–4.03 (cycle, CH, N–CH<sub>2</sub>–P, P–CH<sub>2</sub>–C, bm, 33H); 6.66 (CH, m, 1H); 6.82–6.84 (CH, bm, 3H); 6.97 (CH, d, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 7.12–7.23 (CH, bm, 3H); 7.29 (CH, d, 2H, <sup>3</sup>J<sub>HH</sub> = 9 Hz); 7.34 (CH, d, 1H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 8.03 (d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); <sup>31</sup>P{<sup>1</sup>H}  $\delta$  14.8 (HO–P–OH, d, 1P, <sup>2</sup>J<sub>PP</sub> = 8 Hz); 28.9 (P–CH<sub>2</sub>–N, m, 1P); 32.1 (P–CH<sub>2</sub>–N, m, 1P); 36.2 (P–CH<sub>2</sub>–C, d, 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz).

**MS:** (–) 1323.8 [M–H<sup>+</sup>]<sup>–</sup>. (+) 1369.7 [M–H<sup>+</sup>+2Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.3.



Compound **35**·2H<sub>2</sub>O (108 mg; 150 μmol; 1.0 equiv.) and *N*-hydroxysuccinimide (NHS; 69 mg; 600 μmol; 4.0 equiv.) were dissolved in aqueous acetate buffer HOAc/NaOAc (pH 5.5; 0.5 M; 3 mL). To this solution, EDC·HCl (186 mg; 1.20 mmol; 8 equiv.) in the same buffer (1 mL) was added. Carboxylate functions were activated for 30 min and then pH of this solution was adjusted to 9 using aqueous NaOH (0.05 M). Solution of methyltetrazinamine hydrochloride (78.4 mg; 330 μmol; 2.2 equiv.) in dry DMSO (2.0 mL) was then added and the pH was re-adjusted to 8 using aqueous NaOH (0.05 M). The mixture was stirred at room temperature for three hours. The mixture was then concentrated to 2 mL volume and subsequently purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN). Fraction with product was directly lyophilized. Product was obtained as a violet substance (121 mg; 75 %; 1 step; based on **35**·2H<sub>2</sub>O).

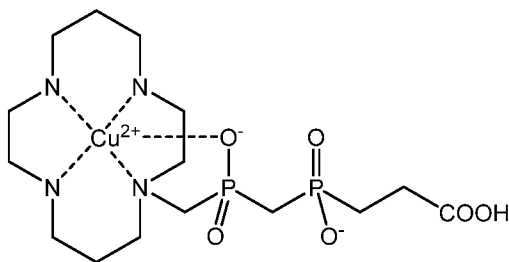
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.82 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 1.88 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 2H); 1.99 (P—CH<sub>2</sub>—C, m, 4H); 2.13 (P—CH<sub>2</sub>—P, m, 4H); 2.34 (CH<sub>3</sub>, s, 6H); 2.40 (CH<sub>2</sub>—CO, t, 4H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.72–3.22 (*cycle*, bm, 20H); 3.34 (N—CH<sub>2</sub>—P, m, 4H); 4.31 (CH<sub>2</sub>—NH—CO, s, 4H); 7.11 (CH, d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); 8.11 (CH, d, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 19.4 (CH<sub>3</sub>, s); 22.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 28.0 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 93 Hz); 29.3 (CH<sub>2</sub>—CO, s); 35.5 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 79 Hz, <sup>1</sup>J<sub>CP</sub> = 75 Hz); 44.2 (CH<sub>2</sub>—NH—CO, s); 50.1 (*cycle*, s); 54.2 (*cycle*, s); 55.8 (*cyclus*, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz); 56.2 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 102 Hz); 58.2 (*cycle*, s); 59.0 (*cycle*, d, 3); 122.6 (*arom.*, s); 127.3 (*arom.*, s); 129.6 (*arom.*, s); 138.2 (*arom.*, s); 161.2 (*arom.*, s); 164.2 (*arom.*, s); 174.2 (CO, d, <sup>3</sup>J<sub>CP</sub> = 17 Hz); <sup>31</sup>P{<sup>1</sup>H} δ 27.2 (CH<sub>2</sub>—P—CH<sub>2</sub>, bm, 2P); 36.1 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 2P, <sup>2</sup>J<sub>PP</sub> = 8 Hz).

**MS**: (–) 1048.1 [M–H<sup>+</sup>]<sup>–</sup>. (+) 1050.3 [M+H<sup>+</sup>]<sup>+</sup>; 1072.2 [M+Na<sup>+</sup>]<sup>+</sup>; 1087.6 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 2:1): R<sub>f</sub> = 0.4.

**EA** (C<sub>42</sub>H<sub>64</sub>N<sub>14</sub>O<sub>10</sub>P<sub>4</sub>·1.5H<sub>2</sub>O, M<sub>R</sub> = 1076.0): C 46.6 (46.9); H 6.2 (6.0); N 17.8 (18.2).

Example 56: *Synthesis of copper(II) complex of compound 14*



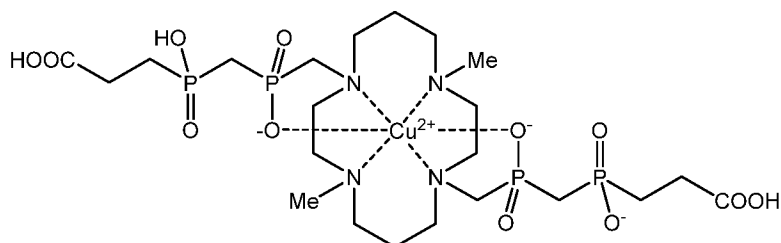
In a glass vial (4 mL), weighted amount of compound **14**·4H<sub>2</sub>O (61.3 mg; 122 μmol; 1.0 equiv.) and Cu(OAc)<sub>2</sub>·H<sub>2</sub>O (34.2 mg; 171 μmol; 1.4 equiv.) was dissolved in aqueous pyridine (2 %; 3 mL) and the mixture was stirred for 10 min. Subsequently, the reaction mixture was evaporated to dryness and then several times co-evaporated with water. The crude complex was purified on ion exchange resin (DOWEX 50; H<sup>+</sup>-forma; water elution). Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in water (50 mL) and the solution was lyophilized. The final product was obtained as dark blue material (51.4 mg; 83 %; 1 step; based on **14**·4H<sub>2</sub>O).

**MS:** (-) 488,0 [M-H<sup>+</sup>]<sup>-</sup>; 524,0 [M+Cl<sup>-</sup>]<sup>-</sup>. (+) 449,0 [M+H<sup>+</sup>]<sup>+</sup>; 512,0 [M+Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH-konc. NH<sub>4</sub>OH-H<sub>2</sub>O 7:3:3): *R<sub>f</sub>* = 0,4.

**EA** (C<sub>15</sub>H<sub>32</sub>N<sub>4</sub>O<sub>6</sub>P<sub>2</sub>Cu·H<sub>2</sub>O, *M<sub>R</sub>* = 508,0): C 35,5 (35,7); H 6,8 (6,6); N 11,0 (11,1).

Example 57: *Synthesis of copper(II) complex of compound 23*

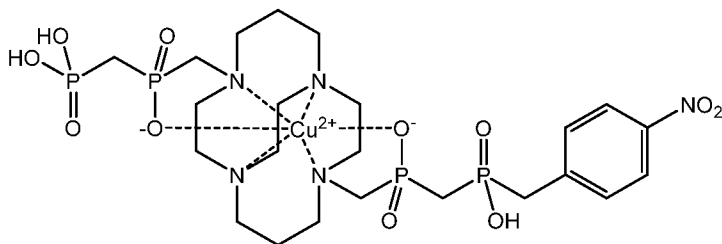


In a glass vial (4 mL), weighted amount of compound **23**·3H<sub>2</sub>O (98.7 mg; 134 μmol; 1.0 equiv.) and Cu(OAc)<sub>2</sub>·H<sub>2</sub>O (41.2 mg; 206 μmol; 1.5 equiv.) was dissolved in aqueous pyridine (5 %; 3 mL) and the mixture was stirred for 10 min. Subsequently, the reaction mixture was evaporated to dryness and then several times co-evaporated with water. The crude complex was purified on ion exchange resin (DOWEX 50; H<sup>+</sup>-forma; water elution). Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in water (100 mL) and the solution was lyophilized. The final product was obtained as dark blue material (83.4 mg; 77 %; 1 step; based on **23**·3H<sub>2</sub>O).

**MS:** (-) 743,2 [M-H<sup>+</sup>]<sup>-</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH-konc. NH<sub>4</sub>OH-H<sub>2</sub>O 7:3:3): *R<sub>f</sub>* = 0,2.

**EA** (C<sub>22</sub>H<sub>45</sub>N<sub>4</sub>O<sub>12</sub>P<sub>4</sub>Cu·0,5HCl·2,5H<sub>2</sub>O, *M<sub>R</sub>* = 808,3): C 29,5 (29,6); H 4,8 (4,9); N 7,8 (7,8).

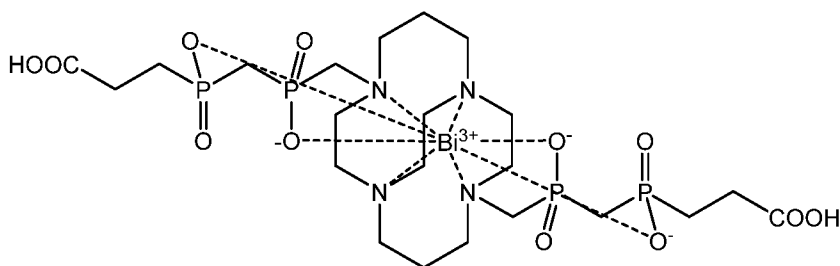
Example 58: *Synthesis of copper(II) complex of compound 37*

In a glass vial (4 mL), weighted amount of compound **37**·4.5H<sub>2</sub>O (29.7 mg; 38.5 μmol; 1.0 equiv.) was dissolved in aqueous solution of CuCl<sub>2</sub> (63 mM; 793 μl; 50.0 μmol; 1.3 equiv.) and the solution pH was slowly adjusted to pH 6 with aqueous solution of NaOH. The crude complex was purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1 % TFA–MeCN). Fraction containing the product was lyophilized. The final product was obtained as light blue material (25.8 mg; 70 %; 1 step; based on **37**·4.5H<sub>2</sub>O).

**MS:** (–) 749,1 [M–H<sup>+</sup>]<sup>–</sup>; 785,0 [M+Cl<sup>–</sup>]<sup>–</sup>. (+) 751,1 [M+H<sup>+</sup>]<sup>+</sup>; 774,1 [M+Na<sup>+</sup>]<sup>+</sup>; 790,2 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–konc. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): *R<sub>f</sub>* = 0,2.

**EA** (C<sub>23</sub>H<sub>41</sub>N<sub>5</sub>O<sub>11</sub>P<sub>4</sub>Cu·0,5TFA·3H<sub>2</sub>O, *M<sub>R</sub>* = 953,5): C 30,2 (30,1); H 5,0 (5,0); N 7,3 (7,4).

15 Example 59: *Synthesis of bismuth(III) complex of compound 35*

In a glass vial (4 mL), weighted amount of compound **35**·2H<sub>2</sub>O (34.4 mg; 47.8 μmol; 1.0 equiv.) was dissolved in aqueous solution of Bi(NO<sub>3</sub>)<sub>3</sub> (50.5 mM; 947 μl; 47.8 μmol; 1.0 equiv.) and the solution pH was slowly adjusted to pH 5 with solid urotropine. The reaction mixture was heated at 90 °C for 2 h. Then, further solid urotropine was added to increase pH to 5. The reaction mixture was heated at 90 °C for 6 h. After cooling, the reaction mixture was evaporated to dryness. The crude complex was purified on ion exchange resin (Amberlite CG50; H<sup>+</sup>-forma; water elution). Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in

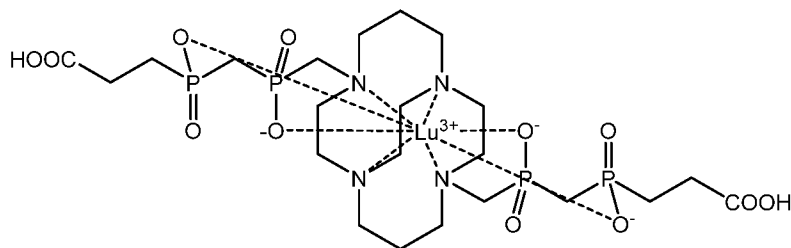
water (50 mL) and the solution was lyophilized. The final product was obtained as white material (34.3 mg; 75 %; 1 step; based on  $35 \cdot 2H_2O$ ).

**MS:** (-) 887,2  $[M-H]^+$ . (+) 889,2  $[M+H]^+$ .

**TLC** ( $SiO_2$ , *i*PrOH-konc.  $NH_4OH-H_2O$  7:3:3):  $R_f = 0,4$ .

5 **EA** ( $C_{22}H_{43}N_4O_{12}P_4Bi \cdot 2H_2O$ ,  $M_R = 955,5$ ): C 28,6 (28,5); H 5,1 (4,9); N 6,1 (6,3).

Example 60: *Synthesis of lutetium(III) complex of compound 35*



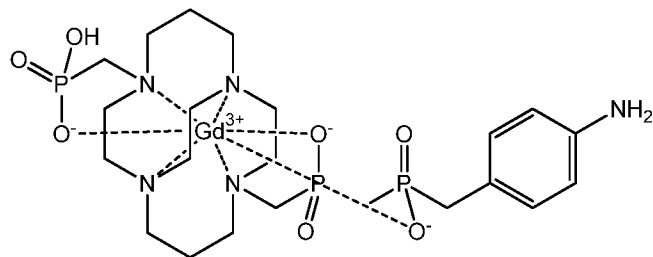
In a glass vial (4 mL), weighted amount of compound  $35 \cdot 2H_2O$  (44.1 mg; 61.4  $\mu$ mol; 1.0 equiv.) was dissolved in aqueous solution of  $LuCl_3$  (50.1 mM; 1.23 ml; 61.6  $\mu$ mol; 1.0 equiv.) and the solution pH was slowly adjusted to pH 7 with aqueous NaOH. The reaction mixture was heated at 80 °C overnight. Next day, the reaction mixture was evaporated to dryness and the crude complex was purified on ion exchange resin (Amberlite CG50;  $H^+$ -forma; water elution). Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in water (50 mL) and the solution was lyophilized. The final product was obtained as white material (37.1 mg; 64 %; 1 step; based on  $35 \cdot 2H_2O$ ).

15 **MS:** (-) 853,1  $[M-H]^+$ . (+) 855,1  $[M+H]^+$ ; 893,1  $[M+K]^+$ .

**TLC** ( $SiO_2$ , EtOH-konc.  $NH_4OH$  1:1):  $R_f = 0,6$ .

20 **EA** ( $C_{22}H_{43}N_4O_{12}P_4Lu \cdot 5H_2O$ ,  $M_R = 944,5$ ): C 28,0 (28,0); H 5,7 (5,9); N 5,9 (5,9).

Example 61: *Synthesis of gadolinium(III) complex of compound 43*



In a glass vial (4 mL), weighted amount of compound  $43 \cdot 3H_2O$  (62.5 mg; 66.2  $\mu$ mol; 1.0 equiv.) was dissolved in aqueous solution of  $GdCl_3$  (49.8 mM; 1.33 ml; 66.2

μmol; 1.0 equiv.) and the solution pH was slowly adjusted to pH 6. The reaction mixture was heated at 80 °C for 2 h. Then, pH was increased to 5 with aqueous NaOH and the reaction mixture was heated at 80 °C overnight. Next day, the reaction mixture was evaporated to dryness and the crude complex was purified on ion exchange resin (Amberlite CG50; H<sup>+</sup>-forma; water elution followed with 10 % AcOH in mixture of water:EtOH 1:1). Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in water (50 mL), evaporated to dryness and dissolved in water again (50 mL) and the solution was lyophilized. The final product was obtained as white material (37.1 mg; 75 %; 1 step; based on **43**·3H<sub>2</sub>O).

**MS:** (-) 853,1 [M-H<sup>+</sup>]<sup>-</sup>. (+) 855,1 [M+H<sup>+</sup>]<sup>+</sup>; 893,1 [M+K<sup>+</sup>]<sup>+</sup>.

Example 62: Comparative experiment: *Labelling of the compounds with <sup>64</sup>Cu*

Aqueous solution of a compound to be labelled (1.0 mM; 1.0 μL) was added to aqueous buffer solution MES-NaOH (1.0 M; pH 6.2; 10.0 μL) in Eppendorf plastic microvial (1.5 mL). The solution was incubated with mild agitation (750 oscillations/min) at 25 °C for 10 min. To the mixture, freshly prepared [<sup>64</sup>Cu]CuCl<sub>2</sub> solution in 10 mM aqueous HCl (6 μL; 9–10 MBq) was added. Then, the reaction mixture was incubated with mild agitation (750 oscillations/min) at 25 °C for 10 min. After 10 min, the reaction mixture was analysed by TLC (SiO<sub>2</sub> 60 W F<sub>254</sub> S; aqueous EDTA-NaOH buffer, pH 5; *R<sub>f</sub>*(free copper) = 0.8–0.9; *R<sub>f</sub>*(complex) = 0). Examples of radiochemical yields (determined by RitaStar; Raytest) of selected compounds involving also examples of currently used ligands (in italics) are given below (for each ligand, labelling was carried out with three different batches-eluates of [<sup>64</sup>Cu]CuCl<sub>2</sub>):

Compound	Radiochemical yield
<b>1</b>	71±10%
<b>8</b>	76±14%
<b>14</b>	83±8%
<b>19</b>	83±9%
<b>32</b>	83±3%
<b>34</b>	62±12%
<b>41</b>	66±8%
<i>NOTA</i>	18±11%

<i>DOTA</i>	9±7%
<i>cb-TE2A</i>	2±1%

Radiochemical yields for the compounds which are subject of the patent application were, under identical conditions, several times better than those of commercial ligands as NOTA, DOTA a *cb-TE2A*.

Example 63: *Labelling of compound 34 with <sup>177</sup>Lu*

Aqueous solution of the non-carrier-added [<sup>177</sup>Lu]LuCl<sub>3</sub> in 0.01 M HCl (50 µL, ca. 12 MBq activity) was added to aqueous solution of NH<sub>4</sub>OAc (0.2 mL of the buffer; pH 5.5; 1 M) in a 1-mL vial. To the solution, a solution of the compound **34** in ultrapure water was added (40 µL, *c* = 50 µM) and the solution was agitated at 90 °C for 20 min. ITLC analysis (1 M aqueous NH<sub>4</sub>OAc : MeOH; 1:1 v/v) confirms presence of the radiochemically pure labelled complex (complex with *R<sub>f</sub>* ~0.9; free <sup>177</sup>Lu has *R<sub>f</sub>* = 0).

Example 64: *Labelling of compound 37 with <sup>213</sup>Bi*

Aqueous solution of NaOAc (pH 5.5; 1 M; 1.6 mL) was added to the non-carrier-added <sup>213</sup>Bi<sup>3+</sup> present as a solution in 0.15 M aqueous KI (1.4 mL) with a nominal activity ca. 150 MBq (i.e. ca. 107 MBq/mL). To the solution of compound **37** in ultrapure water (10 µL, *c* = 100 µM), a solution containing [<sup>213</sup>Bi]Bi<sup>3+</sup> (90 µL, ca. 10 MBq) was added and the solution was incubated at 70 °C for 10 min. Paper liquid chromatography (0.1 M aqueous Na-citrate) showed a quantitative complex formation (*R<sub>f</sub>*(complex) <0.5, <sup>213</sup>Bi-citrate moves with the solvent front).

Example 65: *Synthesis of targeting conjugate of the compound 39 and the Rituximab antibody*

Water used to prepare reaction solutions and used for purifications in the Example is deionized water which was purified from possible metal impurities by ion exchange on Chelex 100 chelating resin. Throughout the procedure, sterile glassware and plastics was used, used commercial chemicals have the highest available purity in a view of metal ion content.

Aqueous solution (4 mL) of Rituximab antibody (0.14 µmol) having concentration 5.0 mg/mL was concentrated by ultrafiltration in MWCO-30 kDa Vivaspin 6 vial to 1 mL (final concentration 20 mg/mL). This concentrated antibody solution was incubated with

a solution of compound **39**·3.5H<sub>2</sub>O (1.1 mg; 1.4 μmol, antibody:ligand 1:10 molar ratio) in anhydrous DMSO (200 μl; dissolved under sonification, sterilized by filtration through syringe microfilter; Millipore, 0.22 μm). Immediately after the reaction mixture preparation, the solution pH was adjusted to 8.2–8.6 with 0.1 M aqueous NaOH solution.

5 The reaction mixture was agitated (Vortex, 450 rev./min.) at room temperature for 2 h. One hour after start of the reaction, pH was re-adjusted to 8.2–8.6 (0.1 M aqueous NaOH). The conjugation was terminated by TRIS-HCl buffer solution (1.0 M; pH 8.8; 50 μL) addition. The conjugate solution was purified from unreacted ligand and other low-molecular-weight impurities by triplicate ultrafiltration with utilization of Vivaspin 6. The

10 ultrafiltration was carried out by the following procedure. The mixture was transferred into washed sterilized Vivaspin 6 vial and the solution volume was adjusted to 6 mL with 0,1 M PBS buffer (pH 8.5). Then, upper part of the Vivaspin vial was incubated in Vortex (1000 oscillations/min) for 5 min. Then, the solution was centrifugated at 4000 rev./min for 45 min to get 0.3 mL solution volume in the upper part of the Vivaspin vial. The

15 antibody solution in the upper part of the vial was adjusted to 6 mL with 0,05 M aqueous NH<sub>4</sub>OAc (pH 7.0), and the step vortexing-centrifugation was repeated (carried out 2-times). After the third centrifugation, the solution containing the antibody was added with water to get the final volume 3 mL. The conjugate purity was checked by spectrophotometry at 280 nm and also by HPLC (UV detection, 280 nm); degradation test

20 and test of the antibody significant losses. The purified conjugate must be stored in the fridge at 4–8 °C temperature. An average number of the chelates per the antibody molecule was determined by MS-MALDI/TOF to be 3.

Example 66: *Labelling of targeting conjugate of the compound 39 and the Rituximab*

25 *antibody with 64-Cu*

The conjugate solution from the Example 65 (0.5 mL) was transferred into Eppendorf plastic microvial (volume 1.5 mL) and, if necessary, its final volume (taking into account also the added radionuclide solution) was adjusted to 1 mL with 0.5 M NH<sub>4</sub>OAc (pH 6.0). The solution was incubated with mild agitation (450 oscillations/min)

30 at 25 °C for 15 min. To the mixture, non-carrier-added radiocopper [<sup>64</sup>Cu]CuCl<sub>2</sub> solution in 0.01 M aqueous HCl (0.12 mL; 200 MBq [<sup>64</sup>Cu]CuCl<sub>2</sub>) was added. Subsequently, solution pH was checked and was found to be 6.0. Then, the reaction mixture was incubated with mild agitation (450 oscillations/min) at 40 °C for 20 min. Once incubation

was finished, the labelled conjugate was sterilized by filtration through syringe microfilter (Millipore; 0,22  $\mu\text{m}$ ). ITLC analysis (1 M aqueous  $\text{NH}_4\text{OAc}$  and MeOH; 1:1 v/v) carried out after addition of aqueous DTPA (to bind free  $^{64}\text{Cu}$ ) to the sterile sample showed radiochemically pure labelled conjugate ( $R_f = 0$ ) and no free  $^{64}\text{Cu}$  (Cu-DTPA,  $R_f \sim 0,9$ ).

Example 67: *Synthesis of conjugate of the compound 39 and the PAMAM dendrimer of the second generation*

Commercial form of the G2-PAMAM dendrimer (16 free amino groups; 20 % solution in MeOH; 0.15 mL; 10  $\mu\text{mol}$ ) was dissolved in  $\text{H}_2\text{O}$  (5 mL) and the compound **39** (127 mg; 160  $\mu\text{mol}$ ; equivalent amount per amino groups on the dendrimer) was dissolved in this solution. Solution pH was carefully adjusted to 9 with aqueous KOH solution and the reaction mixture was stirred at 40  $^\circ\text{C}$  for 12 h. Then, compound **39** (63 mg; 0,5 molar equivalent per dendrimer amino groups) was added again, pH was re-adjusted to 9 aqueous KOH solution and the solution was stirred at 40  $^\circ\text{C}$  for 12 h. Then, the solution was cooled down, solution pH was decreased to pH = 7 and the solution was filtrated through syringe microfilter (Millipore; 0.22  $\mu\text{m}$ ) into ultrafiltration cell. Ultrafiltration (under 3 atm pressure, 3-kDa membrane cut-off) was carried out three times from volume 50 mL to the retenate volume 10 mL, and it was followed by continuous ultrafiltration (4 atm) until 800 ml of the filtrate was obtained. The retenate was lyophilized to give white solids (167 mg; with the active compound content 72 %, the yield is 83 % based on the starting dendrimer). A number of ligand moieties in the dendrimer conjugate ( $^1\text{H}$  NMR spectrum) was estimated to be 15.5.

NMR ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1,88–3,72 ( $\text{CH}_2$ , bm, 39,44H); 7,11–7,33 (CH, bm, 4,00H);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  19,0 (HO—P—OH, bm, 1P); 23,8 (P— $\text{CH}_2$ —P, bm, 1P); 28,2 (P— $\text{CH}_2$ —P, bm); 35,8 (P— $\text{CH}_2$ —P, bm, 1P).

EA ( $M_w$  of the pure dendrimer conjugate  $\sim 14482$ ): 31.4% C, 8.0% H, 9.6%N, 2.5% S; i.e. dendrimer conjugate content was 72 %.

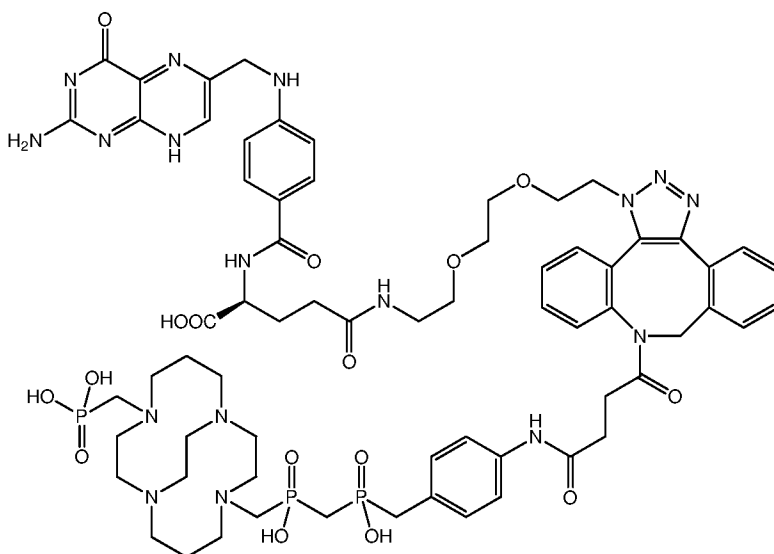
Example 68: *Labelling of targeting conjugate of the compound 39 and the PAMAM dendrimer of the second generation with  $^{64}\text{Cu}$*

The conjugate from Example 67 (5 mg, ca. 0.25  $\mu\text{mol}$ , ca. 3.9  $\mu\text{mol}$  of the macrocycle) was dissolved in ultrapure water (50  $\mu\text{L}$ ) in an Eppendorf plastic microvial

(0.5 mL) and aqueous buffer HOAc/NaOAc (0.5 M, pH 5.8; 0.2 mL) was added. To the mixture, non-carrier-added [ $^{64}\text{Cu}$ ]CuCl<sub>2</sub> in 0.01 M aqueous HCl (120  $\mu\text{L}$ , 180–200 MBq [ $^{64}\text{Cu}$ ]CuCl<sub>2</sub>) was added. Subsequently, solution pH was checked and was found to be 5.8. Then, the reaction mixture was incubated with mild agitation (450 oscillations/min) at 50 °C for 20 min. It led to a solution containing the labelled dendrimer. The product was analysed by thin-layer chromatography (Instant Thin-Layer Chromatography ITLC) on plates with impregnated glass fibres as stationary phase and with 10 mM EDTA in 0.1 M aqueous NH<sub>4</sub>OAc (pH 5.5) as a mobile phase ( $R_f = 0\text{--}0.1$ ) and radioactivity was detected only in the spot of the dendrimer.

10

Example 69: *Synthesis of targeting conjugate with folic acid, based on conjugate 48*

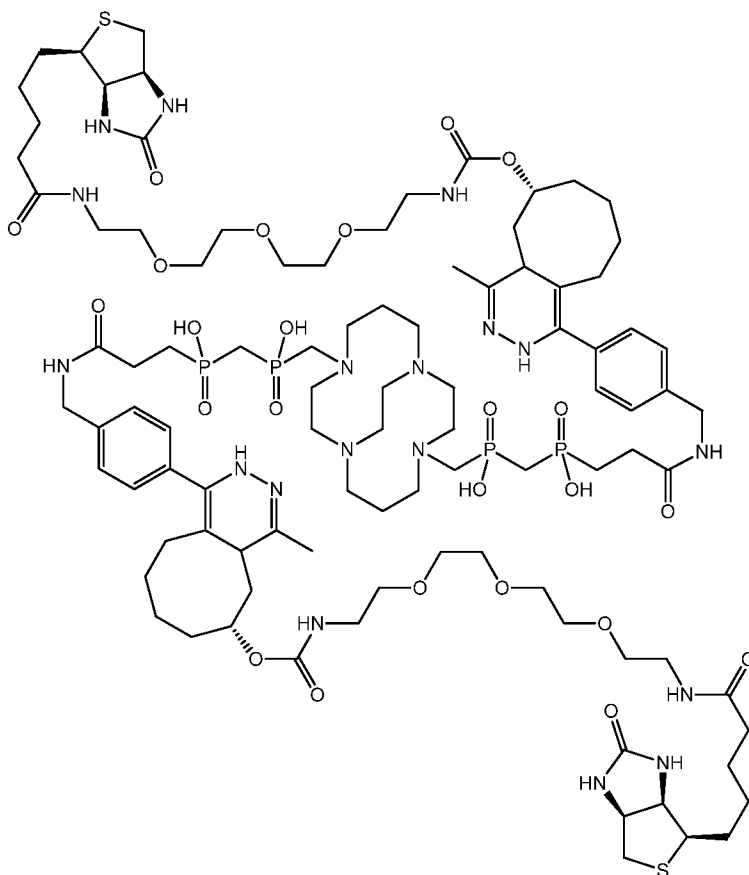


In a glass vial (4 mL), the weighted amount of conjugate **48**·2.5 H<sub>2</sub>O (11 mg; 12  $\mu\text{mol}$ ; 1.0 equiv.) was dissolved in aqueous NH<sub>4</sub>OAc buffer (500 mM; pH 5.0; 0.5 mL). To the solution, freshly prepared solution of  $\delta$ -(1-azido-3,6-dioxaoctan-8-amide) of folic acid (8.6 mg; 14.4  $\mu\text{mol}$ ; 1.2 equiv.) in water (0.3 mL) was added. The mixture was stirred at room temperature for 4 h. The crude product was purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN). Fraction with product was lyophilized. The final product was obtained as a mixture of regioisomers as a white substance (11.4 mg).

20

**MS:** (+) 1467.6 [M+H]<sup>+</sup>, 1489.2 [M+Na]<sup>+</sup>.

Example 70: *Synthesis of targeting conjugate with biotin, based on conjugate 49*

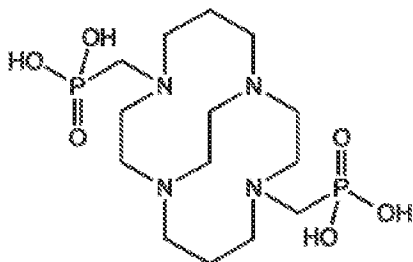


In a glass vial (4 mL), the weighted amount of conjugate **49**·1.5 H<sub>2</sub>O (11.8 mg; 11 μmol; 1.0 equiv.) was dissolved in water, NH<sub>4</sub>OAc (500 mM; pH 5.0; 0.5 mL). To the solution, freshly prepared solution of commercial biotin derivative TCO-PEG3-biotin (7.5 mg; 13.2 μmol; 1.2 equiv.) in water (0.4 mL) was added. The mixture was stirred at room temperature for 30 min. The crude product was purified by preparative HPLC (C8; gradient elution H<sub>2</sub>O–0.1% TFA–MeCN). Fraction with product was lyophilized. The final product was obtained as a mixture of regioisomers as a white substance (15.2 mg).

**MS:** (+) 1590,5 [M+H<sup>+</sup>]<sup>+</sup>, 1612,8 [M+Na<sup>+</sup>]<sup>+</sup>.

Example 71: Comparative experiment: *Labelling of compounds according to the present invention and the compound cb-TE2P, representing the state-of-the-art, with <sup>64</sup>Cu*

Structural formula of *cb-TE2P*



Aqueous solution of a compound to be labelled (1.0 mM; 1.0  $\mu$ L) was added to aqueous buffer solution MES-NaOH (1.0 M; pH 5.5; 10.0  $\mu$ L) in Eppendorf plastic microvial (1.5 mL). The solution was incubated with mild agitation (750 oscillations/min) at 25  $^{\circ}$ C for 10 min. To the mixture, freshly prepared [ $^{64}$ Cu]CuCl<sub>2</sub> solution in 10 mM aqueous HCl (6  $\mu$ L; 9–10 MBq) was added. Then, the reaction mixture was incubated with mild agitation (750 oscillations/min) at 25  $^{\circ}$ C for 60 min. After 60 min, the reaction mixture was analysed by TLC (SiO<sub>2</sub> 60 W F<sub>254</sub> S; aqueous EDTA-NaOH buffer, pH 5;  $R_f$ (free copper) = 0.8–0.9;  $R_f$ (complex) = 0). Examples of radiochemical yields (determined by RitaStar; Raytest) of the selected compounds involving also examples of currently used ligands (in italics) are given below (for each ligand, labelling was carried out with at least three different batches-eluates of [ $^{64}$ Cu]CuCl<sub>2</sub>). The same procedure was used at pH 6.2, but the reaction mixture was incubated only 10 min. Results are summarized in Table 1.

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Table 1: Radiochemical yield (%) in labelling of compounds of the present invention and their analogues at two pH values.

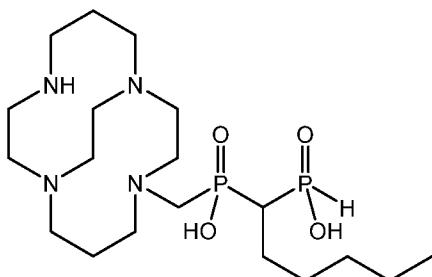
LIGAND	pH 6.2	pH 5.5
<b>8</b>	76 $\pm$ 14	79
<b>32</b>	83 $\pm$ 3	82
<b>37</b>	90 $\pm$ 13	99
NOTA	18 $\pm$ 11	26
DOTA	9 $\pm$ 7	35
<i>cb</i> -TE2A	2 $\pm$ 1	1
<i>cb</i> -TE2P	46 $\pm$ 7	5

Radiochemical yield for compounds which are subject of the present invention at pH 6.2 significantly surpasses those of the state-of-the-art ligands (NOTA, DOTA, *cb*-TE2A, *cb*-TE2P). In the labelling at pH 5.5 (it was chosen as, at this pH, there is a smaller

20

complexation competition with  $Zn^{2+}$  a  $Ni^{2+}$  which are product of the  $^{64}Cu$  radioactive decay), the difference between claimed compounds and the state-of-the-art ligands is huge.

5 Example 72: *Synthesis of compound 50*



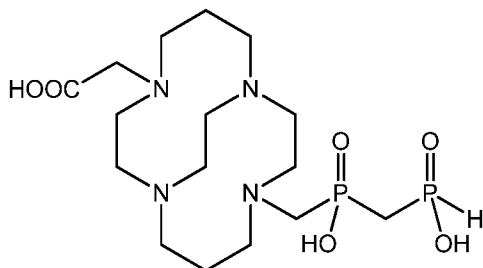
To a glass vial (20 mL), 1,4,8,11-tetraazabicyclo[6.6.2]hexadecan (881 mg; 3.89 mmol; 2.2 equiv.) was weighted. Subsequently, hexan-1,1-diyl-bis(phosphinic acid) (synthesized according to S. Gouault-Bironneau et al. *Org. Lett.* 2005, 7, 5909–5912; 1.14 g; 5.33 mmol; 3.0 equiv.), paraformaldehyd (53.0 mg; 1.77 mmol; 1.0 ekviv.) and aqueous HCl (5 M; 20 mL) were added. The suspension was stirred at 80 °C overnight. Next day, the reaction mixture was evaporated to dryness and the residue was several times co-evaporated with water. The crude product was purified by ion exchange resin chromatography (Amberlite IRA 402;  $OH^-$ -form;  $H_2O \rightarrow 10\%$  aqueous AcOH). Acetic acid fraction with product was evaporated to dryness and then several times co-evaporated with water. The residue was dissolved in water (100 mL) and the solution was lyophilized. The final product was obtained as a white hydroscopic material (412 mg; 52 %; 1 step; based on paraformaldehyde).

**NMR** ( $D_2O$ ):  $^1H$   $\delta$  0.85 ( $CH_3$ , s, 3H) 1.11–2.22 ( $CH_2-CH_2-CH_2-CH_2$ , P—CH—P, cycle, bm, 11H); 2.31 (cycle, m, 1H); 2.35 (cycle, m, 1H); 2.42–3.89 (cycle, N— $CH_2$ —P bm, 22H); 7.09 (PH, m, 1H);  $^{13}C\{^1H\}$   $\delta$  15.0 ( $CH_3$ , s); 16.2 ( $CH_2$ , m); 18.9 (cycle, s); 20.1 (cycle, s); 22.0 ( $CH_2$ , s); 28.3 ( $CH_2$ , s); 30.3 ( $CH_2$ , s); 41.2 (P—CH—P, m); 41.9 (cycle, s); 44.2 (cycle, s); 46.3 (cycle, s); 48.8 (cycle, s); 50.3 ( $CH_2$ —N— $CH_2$ —P, d,  $^3J_{CP} = 8$  Hz); 54.3 (cycle, s); 55.2 (N— $CH_2$ —P, d,  $^1J_{CP} = 91$  Hz); 56.7 (cycle, s); 56.9 (cycle, s); 57.3 (cycle, s); 58.1 (cycle, s);  $^{31}P$   $\delta$  23.2 (PH, dm, 1P,  $^1J_{PH} = 528$  Hz); 29.8 (P— $CH_2$ —N, m, 1P).

**MS:** (–) 451.4  $[M-H^+]^-$ ; 473.4  $[M-2H^+Na^+]^-$ . (+) 453.5  $[M+H^+]^+$ ; 475.4  $[M+Na^+]^+$ ; 497.5  $[M-H^++2Na^+]^+$ .

**EA** ( $C_{19}H_{42}N_4O_4P_2 \cdot 3H_2O$ ,  $M_R = 506.6$ ): C 45.1 (45.4); H 9.6 (9.2); N 11.1 (10.9).



Example 74: *Synthesis of compound 52*

Into a glass vial (20 mL), compound **28**·2.5HCl·H<sub>2</sub>O (230 mg; 468 μmol; 1.0 equiv.) and chloroacetic acid (134 mg; 1.42 mmol; 3.0 equiv.) were weighted, H<sub>2</sub>O (10 mL) and solid LiOH·H<sub>2</sub>O (398 mg; 9.48 mmol; 20 equiv.) were consecutively added and the mixture was heated at 60 °C for 2 days. Subsequently, the reaction mixture was directly purified by ion exchange resin chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O → 10 % aqueous pyridine). Pyridine fraction with product was evaporated to dryness and then several times co-evaporated with water. The residue was dissolved in water (50 mL) and the solution was lyophilized. The final product was obtained as a white material (174 mg; 81 %; 1 step; based on **28**·2.5HCl·H<sub>2</sub>O).

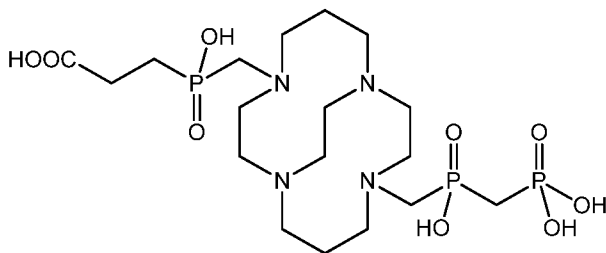
**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.72 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 1.80 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.08 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>PH</sub> = 16 Hz); 2.30 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.30 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.56 (*cycle*, m, 4H); 2.84 (*cycle*, m, 3H); 3.09 (*cycle*, m, 2H); 3.15 (*cycle*, m, 2H); 3.19 (N—CH<sub>2</sub>—P, *cycle*, 2H); 3.27 (*cycle*, m, 3H); 3.36 (*cycle*, m, 2H); 3.63 (*cycle*, m, 1H); 3.75 (N—CH<sub>2</sub>—P, m, 1H); 3.78 (*cycle*, m, 2H); 3.89 (CH<sub>2</sub>—COOH, s, 2H); 7.14 (PH, d, 1H, <sup>1</sup>J<sub>HP</sub> = 535 Hz); <sup>13</sup>C {<sup>1</sup>H} δ 17.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 19.8 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 34.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 85 Hz; <sup>1</sup>J<sub>CP</sub> = 77 Hz); 42.0 (*cycle*, s); 48.1 (*cycle*, s); 49.4 (*cycle*, s); 49.5 (*cycle*, s); 52.1 (CH<sub>2</sub>—N—CH<sub>2</sub>—P, d, <sup>3</sup>J<sub>CP</sub> = 6 Hz); 54.0 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 93 Hz); 54.4 (*cycle*, s); 55.8 (*cycle*, s); 56.8 (CH<sub>2</sub>—COOH); 57.8 (*cycle*, s); 59.2 (*cycle*, s); 60.1 (*cycle*, s); 172.4 (CO, s); <sup>31</sup>P δ 19.9 (PH, dt, 1P, <sup>1</sup>J<sub>PH</sub> = 535 Hz, <sup>2</sup>J<sub>PH</sub> = 16 Hz); 27.8 (P—CH<sub>2</sub>—N, m, 1P).

**MS**: (–) 439.0 [M–H<sup>+</sup>]<sup>–</sup>. (+) 441.1 [M+H<sup>+</sup>]<sup>+</sup>; 463.2 [M+Na<sup>+</sup>]<sup>+</sup>; 479.1 [M+K<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.2.

**EA** (C<sub>16</sub>H<sub>34</sub>N<sub>4</sub>O<sub>6</sub>P<sub>2</sub>·H<sub>2</sub>O, M<sub>R</sub> = 458.4): C 41.9 (41.8); H 7.9 (7.6); N 12.2 (12.2).

## Example 75: Synthesis of compound 53



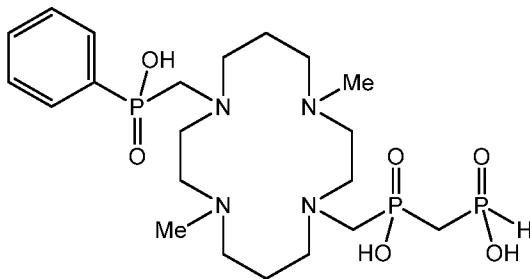
Into a glass vial (4 mL), compound **29**·1.5HCl·0.5H<sub>2</sub>O (98.2 mg; 213 μmol; 1.0 equiv.) was weighted, and 2-carboxyethylphosphinic acid (89 mg; 644 μmol; 3.0 equiv.), paraformaldehyde (19.3 mg; 643 μmol; 3.0 equiv.) and aqueous HCl (6 M; 2 mL) were consecutively added. The suspension was stirred at 80 °C for 2 days. Next day, the reaction mixture was evaporated to dryness and the residue was several times co-evaporated with water. The crude product was purified by ion exchange resin chromatography (DOWEX 50; H<sup>+</sup>-form; H<sub>2</sub>O). The fraction containing a pure product were unified and lyophilized. The final product was obtained as a white material (61.9 mg; 48 %; 1 step; based on **29**·1.5HCl·0.5H<sub>2</sub>O).

<sup>31</sup>P{<sup>1</sup>H} δ 19.8 (HO—P—OH, d, 1P, <sup>2</sup>J<sub>PP</sub> = 9 Hz); 27.3 (CH<sub>2</sub>—P—CH<sub>2</sub>, bm, 1P); 34.1 (CH<sub>2</sub>—P—CH<sub>2</sub>, bm, 1P).

MS: (–) 529.0 [M–(H<sub>3</sub>O)<sup>+</sup>]<sup>–</sup>; 547.1 [M–H<sup>+</sup>]<sup>–</sup>. (+) 549.1 [M+H<sup>+</sup>]<sup>+</sup>; 571.2 [M+Na<sup>+</sup>]<sup>+</sup>; 587.2 [M+K<sup>+</sup>]<sup>+</sup>.

EA (C<sub>18</sub>H<sub>39</sub>N<sub>4</sub>O<sub>9</sub>P<sub>3</sub>·3H<sub>2</sub>O, M<sub>R</sub> = 602.5): C 35.9 (36.3); H 7.5 (7.2); N 9.3 (9.3).

## Example 76: Synthesis of compound 54



Into a glass vial (4 mL), compound **3**·3.5H<sub>2</sub>O (112 mg; 250 μmol; 1.0 equiv.) was weighted, and phenylphosphonic acid (357mg; 2.51 mmol; 10.0 equiv.), paraformaldehyde (15.1 mg; 503 μmol; 2.0 equiv.) and aqueous HCl (12 M; 2 mL) were consecutively added. The suspension was stirred at 60 °C for 3 days. Subsequently, the reaction mixture was evaporated to dryness and the residue was

several times co-evaporated with water. The crude product was purified by preparative HPLC (C8; H<sub>2</sub>O–0.1 % TFA–MeCN). The fraction containing product was lyophilized. The final product was obtained as a white material (104 mg; 59 %; 1 step; based on 3·3.5H<sub>2</sub>O).

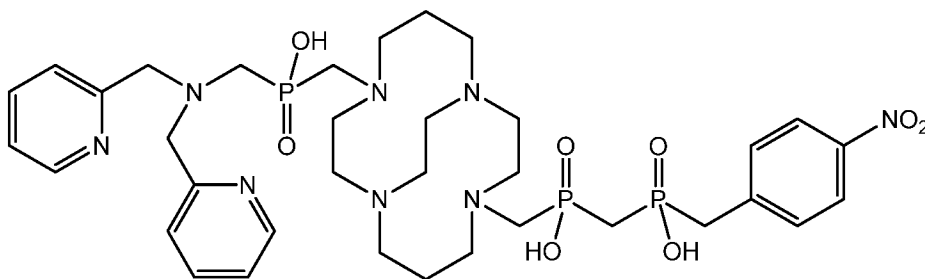
5 **NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.73 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 1.79 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, p, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.03 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz, <sup>2</sup>J<sub>HP</sub> = 15 Hz); 2.28 (CH<sub>3</sub>, s, 3H); 2.33 (CH<sub>3</sub>, s, 3H); 2.44 (cycle, t, 2H, <sup>3</sup>J<sub>HH</sub> = 7 Hz); 2.48 (cycle, m, 4H); 2.71 (cycle, t, 2H, <sup>3</sup>J<sub>HH</sub> = 6 Hz); 2.66–3.16 (cycle, N—CH<sub>2</sub>—P, m, 12H); 7.09 (PH, dm, <sup>1</sup>J<sub>HP</sub> = 534 Hz); 7.48 (arom., m, 2H); 7.55 (arom., m, 1H); 7.73 (arom., m, 2H);  
 10 <sup>13</sup>C{<sup>1</sup>H} δ 23.1 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 23.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 35.9 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 77 Hz, <sup>1</sup>J<sub>CP</sub> = 75 Hz); 43.0 (cycle, s); 43.3 (CH<sub>3</sub>, s); 43.6 (CH<sub>3</sub>, s); 44.5 (cycle, s); 51.0 (cycle, d, <sup>3</sup>J<sub>CP</sub> = 8 Hz); 52.2 (cycle, s); 54.1 (cycle, s); 54.3 (cycle, s); 54.7 (cycle, d, <sup>3</sup>J<sub>CP</sub> = 6 Hz); 55.3 (cycle, s); 55.5 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); 56.9 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 101 Hz); 128.8 (arom., d, <sup>3</sup>J<sub>CP</sub> = 14 Hz); 131.0 (arom., d, <sup>2</sup>J<sub>CP</sub> = 12 Hz); 131.7 (arom., d, <sup>1</sup>J<sub>CP</sub> = 135 Hz); 132.8 (arom., d, <sup>4</sup>J<sub>CP</sub> = 3 Hz); <sup>31</sup>P δ 23.6 (PH, dtm, 1P, <sup>1</sup>J<sub>PH</sub> = 534 Hz, <sup>2</sup>J<sub>PH</sub> = 18 Hz); 26.7 (N—CH—P, m, 1P) 34.2 (N—CH—P, m, 1P).

**MS:** (–) 537.2 [M–H<sup>+</sup>]<sup>–</sup>. (+) 539.4 [M+H<sup>+</sup>]<sup>+</sup>; 561.3 [M+Na<sup>+</sup>]<sup>+</sup>; 577.2 [M+K<sup>+</sup>]<sup>+</sup>.

**EA** (C<sub>21</sub>H<sub>41</sub>N<sub>4</sub>O<sub>6</sub>P<sub>3</sub>·TFA·3H<sub>2</sub>O, M<sub>R</sub> = 706.6): C 39.1 (39.1); H 6.9 (6.6); N 7.9 (8.3).

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Example 77: *Synthesis of compound 55*



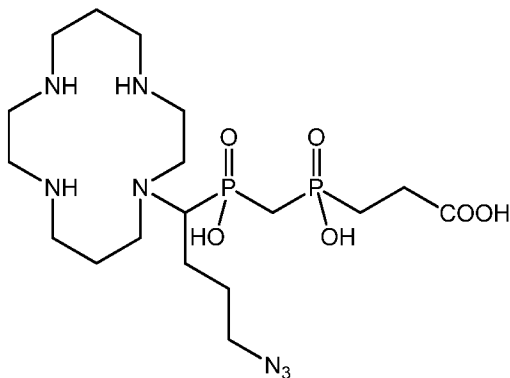
25 Into a glass vial (20 mL), compound 35·5.5H<sub>2</sub>O (185 mg; 300 μmol; 1.0 equiv.) was weighted, and [di(2-picolyl)amino]methylphosphinic acid (synthesized according to J. Yu et al. *J. Am. Chem. Soc.* 2015, **137**, 14173–14179; 200 mg; 721 μmol; 2.4 equiv.), paraformaldehyde (36.4 mg; 1.21 mmol; 4.0 equiv.) and aqueous HCl (6 M; 4 mL) were consecutively added. The suspension was stirred at 80 °C for 2 days. Subsequently, the reaction mixture was evaporated to dryness and the residue was

several times co-evaporated with water. The crude product was purified by preparative HPLC (C8; H<sub>2</sub>O–0.1 % TFA–MeCN). The fraction containing product was lyophilized. The final product was obtained as an off-yellow material (203 mg; 74 %; 1 step; based on **35**·5.5H<sub>2</sub>O).

5 **NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.73 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 1.79 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.08 (P—CH<sub>2</sub>—P, m, 2H); 2.33 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.38 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, m, 1H); 2.63 (*cycle*, bm, 4H); 2.82–3.01 (*cycle*, bm, 5H); 3.07–3.35 (*cycle*, P—CH<sub>2</sub>—C, N—CH<sub>2</sub>—P, bm, 13H); 3.42–3.57 (*cycle*, N—CH<sub>2</sub>—P, bm, 4H); 3.62–3.83 (*cycle*, N—CH<sub>2</sub>—P, bm, 4H); 4.11 (CH<sub>2</sub>—N—CH<sub>2</sub>, s, 4H), 7.44 (*arom.*, m, 6H); 7.96  
10 (*arom.*, t, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); 8.23 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 8 Hz); 8.40 (*arom.*, dm, 2H, <sup>2</sup>J<sub>HH</sub> = 6 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 19.2 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 20.7 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 33.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 85 Hz, <sup>1</sup>J<sub>CP</sub> = 81 Hz); 40.8 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 86 Hz); 42.4 (*cycle*, s); 48.2 (*cycle*, s); 49.5 (*cycle*, s); 50.0 (*cycle*, s); 52.6 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 7 Hz); 54.2 (*cycle*, s); 55.5 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 90 Hz); 57.2 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 97 Hz); 57.5 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 106 Hz); 59.1 (*cycle*, s); 59.7 (*cycle*, s); 60.2 (*cycle*, s); 60.7 (CH<sub>2</sub>—N—CH<sub>2</sub>, s, 4H); 123.8 (CH—C—N, s); 125.3 (*arom.*, s); 126.9 (*arom.*, s); 130.8 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz); 142.7 (*arom.*, s); 144.0 (CH—C—CH<sub>2</sub>, d, <sup>2</sup>J<sub>CP</sub> = 8 Hz); 145.4 (*arom.*, s); 147.0 (*arom.*, s); 155.3 (*arom.*, s); <sup>31</sup>P{<sup>1</sup>H} δ 24.8 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz); 30.8 (CH<sub>2</sub>—P—CH<sub>2</sub>, bs, 1P), 33.1  
20 (CH<sub>2</sub>—P—CH<sub>2</sub>, s, 1P);  
**MS**: (–) 805.2 [M–H]<sup>–</sup>. (+) 807.3 [M+H]<sup>+</sup>; 829.3 [M+Na]<sup>+</sup>; 845.3 [M+K]<sup>+</sup>; 851.3 [M–H<sup>+</sup>+2Na]<sup>+</sup>.  
**EA** (C<sub>35</sub>H<sub>53</sub>N<sub>8</sub>O<sub>8</sub>P<sub>3</sub>·2TFA·H<sub>2</sub>O, M<sub>R</sub> = 1052.8): C 44.5 (44.5); H 5.5 (5.4); N 10.6 (10.4).

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Example 78: *Synthesis of compound 56*



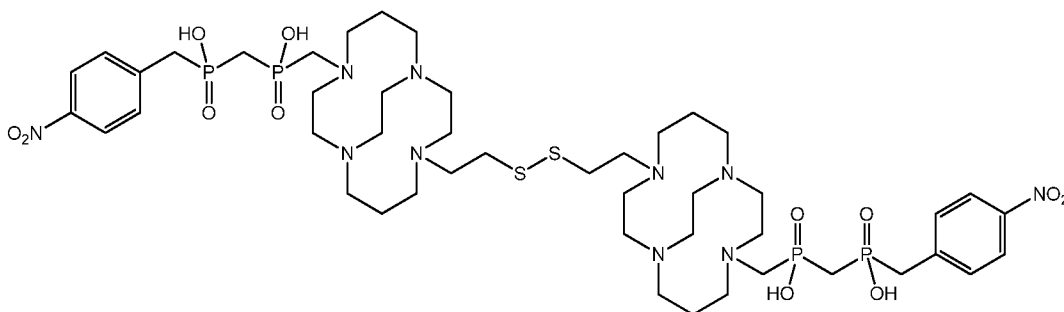
Into a glass vial (20 mL), 1,4,8,11-tetraazacyclotetradecane (463 mg; 2.31 mmol; 4.0 equiv.) was weighted, and, compound **D** (224 mg; 1.04 mmol; 1.8 equiv.), 4-azido-butanal (64.8 mg; 573  $\mu\text{mol}$ ; 1.0 equiv.), and aqueous HCl (6 M; 5 mL) were consecutively added. The suspension was stirred at 60 °C overnight. On next day, the reaction mixture was evaporated to dryness and the residue was several times co-evaporated with water. The crude product was purified by ion exchange resin chromatography (DOWEX 50;  $\text{H}^+$ -form;  $\text{H}_2\text{O} \rightarrow 10\%$  aqueous pyridine). The pyridine fraction containing product was evaporated to dryness and the residue was several times co-evaporated with water. Subsequently, the crude product was purified by preparative HPLC (C8;  $\text{H}_2\text{O}$ -0.1% TFA-MeCN). The fraction containing product was lyophilized. The final product was obtained as a white material (212 mg; 70%; 1 step; based on 4-azido-butanal).

**NMR** ( $\text{D}_2\text{O}$ ):  $^1\text{H}$   $\delta$  1.52 ( $\text{CH}_2\text{—N}_3$ , t, 2H,  $^3J_{\text{HH}} = 7$  Hz); 1.65–2.28 (P— $\text{CH}_2$ —P,  $\text{CH}_2$ , cycle, bm, 12H); 2.44–3.52 (cycle, bm, 19H);  $^{13}\text{C}\{^1\text{H}\}$   $\delta$  19.8 ( $\text{CH}_2$ , s); 22.8 (cycle, s); 24.4 ( $\text{CH}_2$ , s); 26.5 (cycle, s); 28.2 (P— $\text{CH}_2$ —C, d,  $^1J_{\text{CP}} = 93$  Hz); 30.3 ( $\text{CH}_2$ —CO, s); 34.2 (P— $\text{CH}_2$ —P, t,  $^1J_{\text{CP}} = 76$  Hz); 44.2 (cycle, s); 45.3 (cycle, s); 46.8 (cycle, s); 47.0 (cycle, s); 49.5 (cycle, s); 49.8 (cycle, d,  $^3J_{\text{CP}} = 12$  Hz); 50.0 (cycle, s); 50.5 (cycle, s); 52.2 ( $\text{CH}_2\text{—N}_3$ , s); 53.1 (N—CH—P, d,  $^1J_{\text{CP}} = 109$  Hz); 177.2 (CO, d,  $^3J_{\text{CP}} = 14$  Hz);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  28.4 (P— $\text{CH}_2$ —C, d, 1P,  $^2J_{\text{PH}} = 12$  Hz); 34.4 (N—CH—P, m, 1P).

**MS**: (–) 492.2 [ $\text{M}-(\text{H}_3\text{O})^+$ ] $^-$ ; 510.2 [ $\text{M}-\text{H}^+$ ] $^-$ .

**EA** ( $\text{C}_{18}\text{H}_{39}\text{N}_7\text{O}_6\text{P}_2 \cdot \text{H}_2\text{O}$ ,  $M_{\text{R}} = 529.5$ ): C 40.8 (50.0); H 7.8 (7.7); N 18.5 (18.6).

#### Example 79: Synthesis of compound **57**



25

Into a glass vial (20 mL),  $35 \cdot 5.5\text{H}_2\text{O}$  (145 mg; 235  $\mu\text{mol}$ ; 4.0 equiv.) and bis(2-bromoethyl)disulphide (16.5 mg; 59  $\mu\text{mol}$ ; 1.0 equiv.) were weighted, and dry MeCN (10 mL) and anhydrous  $\text{K}_2\text{CO}_3$  (163 mg; 1.18 mmol; 20 equiv.) were consecutively added. The mixture was stirred at room temperature for 5 days. Subsequently, the

mixture was filtered through syringe filter (Millipore; 0.22  $\mu\text{m}$ ). The filtrate was purified by preparative HPLC (C8;  $\text{H}_2\text{O}$ –0.1% TFA–MeCN). The fraction containing product was lyophilized. The final product was obtained as an off-yellow material (58.5 mg; 69 %, 1 step, based on bis(2-bromoethyl)disulphide).

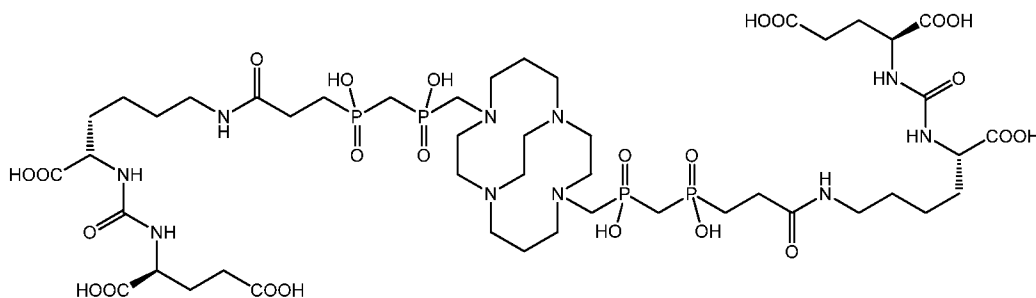
5  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  30.4 ( $\text{CH}_2\text{—P—CH}_2$ , bm, 2P); 33.2 ( $\text{CH}_2\text{—P—CH}_2$ , m, 2P).

MS: (–) 1173.5  $[\text{M} - 2\text{H}^+ + \text{Na}^+]^-$ . (+) 1152.6  $[\text{M} + \text{H}]^+$ ; 1175.7  $[\text{M} + \text{Na}]^+$ ; 1191.6  $[\text{M} + \text{K}]^+$ ; 1197.6  $[\text{M} - \text{H}^+ + 2\text{Na}]^+$ ; 1219.7  $[\text{M} - 2\text{H}^+ + 3\text{Na}]^+$ .

EA ( $\text{C}_{46}\text{H}_{80}\text{N}_{10}\text{O}_{12}\text{P}_4\text{S} \cdot 2\text{TFA} \cdot 3\text{H}_2\text{O}$ ,  $M_{\text{R}} = 1435.3$ ): C 41.8 (41.4); H 6.2 (5.9); N 9.8 (9.6).

10 Disulphidic (oxidized) form of thiols is a stable form suitable for storage and the thiol group could be easily generated from them by *in-situ* reaction with mild reductive agents as tris(2-carboxyethyl)phosphine (TCEP).

15 Example 80: Conjugate of compound 35 with a compound targeting the PSMA receptor



20 Into a glass vial, compound  $35 \cdot 2\text{H}_2\text{O}$  (79.0 mg; 110  $\mu\text{mol}$ ; 1.0 equiv.) was weighed. The following reagents were added in the consecutive order: (*S*)-di-*t*-butyl 2-{3-[(*S*)-6-amino-1-*t*-butoxy-1-oxohexan-2-yl]ureido}pentanedioate (prepared according to R. P. Murelli et al. *J. Am. Chem. Soc.* 2009, **131**, 17090–17092; 108 mg; 220  $\mu\text{mol}$ ; 2.2 equiv.), dry DMSO (2 mL), *N,N*-diisopropylethylamine (96  $\mu\text{L}$ ; 550  $\mu\text{mol}$ ; 5.0 equiv.) and TBTU (142 mg; 442  $\mu\text{mol}$ ; 4.0 equiv.). After stirring at room temperature for 30 min, another *N,N*-diisopropylethylamine (96  $\mu\text{L}$ ; 550  $\mu\text{mol}$ ; 5.0 equiv.) and TBTU (142 mg; 442  $\mu\text{mol}$ ; 4.0 equiv.) were added. After stirring for further 30 min, the mixture was directly purified by preparative HPLC (C8; gradient elution  $\text{H}_2\text{O}$ –0.1 % TFA–MeCN). The fraction containing product was evaporated to dryness. The residue was dissolved in trifluoroacetic acid (2 mL) and the solution was stirred room temperature overnight. Then, the mixture was evaporated to dryness and

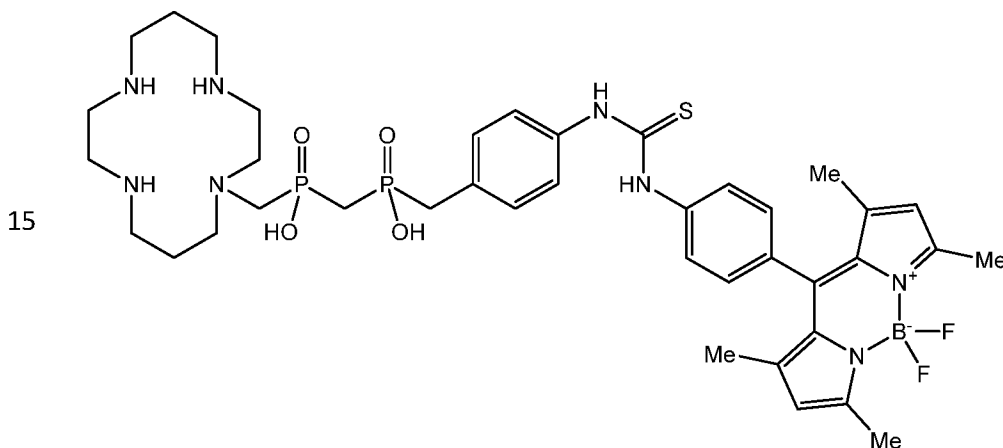
co-evaporated with water several times. The residue was dissolved in water (50 mL) and lyophilized. The final product was obtained as a white material (79.2 mg; 54 %; based on  $35 \cdot 2H_2O$ ).

**NMR** ( $D_2O$ ):  $^1H$   $\delta$  1.45 ( $CH_2$ , m, 4H); 1.57 ( $CH_2$ , p, 4H,  $^3J_{HH} = 5$  Hz); 1.68 ( $CH_2$ , m, 4H); 1.76–2.33 (*cycle*,  $CH_2$ , P– $CH_2$ –C bm, 10H); 2.39 ( $CH_2$ –CO, t, 4H,  $^3J_{HH} = 8$  Hz); 2.61–3.92 (*cycle*,  $CH_2$ –N–CO, N– $CH_2$ –P, bm, 28H); 4.36 ( $CH$ , t, 2H,  $^3J_{HH} = 7$  Hz); 4.52 ( $CH$ , t, 2H,  $^3J_{HH} = 6$  Hz);  $^{31}P\{^1H\}$   $\delta$  28.3 ( $CH_2$ –P– $CH_2$ , bm, 2P); 36.0 ( $CH_2$ –P– $CH_2$ , m, 2P).

**MS**: (–) 1284.2 [ $M-H^+$ ] $^-$ ; (+) 1286.5 [ $M+H^+$ ] $^+$ ; 1307.0 [ $M+Na^+$ ] $^+$ ; 1323.3 [ $M+K^+$ ] $^+$ .

**EA** ( $C_{46}H_{84}N_{10}O_{24}P_4 \cdot 2.5H_2O$ ,  $M_R = 1330.1$ ): C 41.0 (41.4); H 6.9 (6.7); N 10.1 (10.5). The conjugates with other PSMA inhibitors, based on urea derivatives or on organophosphorus acids, can be obtained analogously.

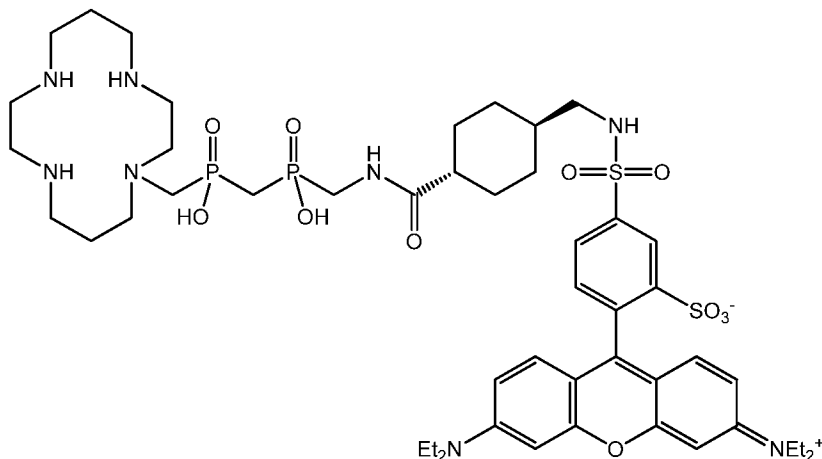
Example 81: Conjugate of compound **9** with a fluorescence label, BODIPY



Into a glass vial,  $9 \cdot 3H_2O$  (21.7 mg; 42.1  $\mu$ mol; 2.5 equiv.) and –NCS derivative of phenyl-BODIPY (6.4 mg; 16.8  $\mu$ mol; 1.0 equiv.) were weighted out and MeCN (250  $\mu$ L) and aqueous buffer solution (MOPS-NaOH; 0.3 M; pH 8, 550  $\mu$ L) were consecutively added. The mixture was stirred at room temperature overnight. Subsequently, the mixture was directly purified by preparative HPLC (C8;  $H_2O$ –0.1% TFA–MeCN). The fraction containing product was lyophilized. The final product was obtained as an orange material (10.3 mg; 73 %, 1 step, based on NCS derivative of bodipy).

**NMR** ( $D_2O$ ):  $^{31}P\{^1H\}$   $\delta$  30.1 (P– $CH_2$ –N, m, 1P); 31.8 (P– $CH_2$ –N, s, 1P).

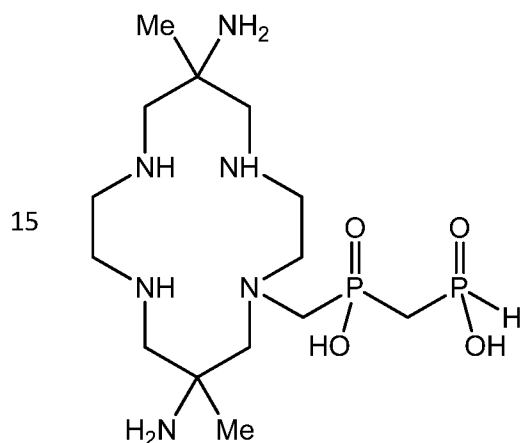
**MS**: (–) 841.2 [ $M-H^+$ ] $^-$ ; (+) 843.3 [ $M+H^+$ ] $^+$ ; 865.3 [ $M+Na^+$ ] $^+$ ; 881.4 [ $M+K^+$ ] $^+$ .

Example 82: Conjugate of compound **13** with rhodamine

Into a glass vial, **13**·2.5H<sub>2</sub>O (10.1 mg; 24.5 μmol; 2.1 equiv.) and *N*-[*trans*-4-(succinimidylloxycarbonyl)cyclohexylmethyl]sulfonamid-disulforhodaminu B (9.3 mg; 11.7 μmol; 1.0 equiv.) were weighted out and MeCN (500 μL) and aqueous buffer solution (MES-NaOH; 0.5 M; pH 7, 500 μL) were consecutively added. The mixture was stirred at room temperature two days. Subsequently, the mixture was directly purified by preparative HPLC (C8; H<sub>2</sub>O–0.1% TFA–MeCN). The fraction containing product was lyophilized. The final product was obtained as a pink material (5.2 mg; 45%, 1 step, based on NCS ester of rhodamine B).

**NMR** (D<sub>2</sub>O): <sup>31</sup>P{<sup>1</sup>H} δ 29.8 (*P*–CH<sub>2</sub>–N, bm, 1P); 36.2 (*P*–CH<sub>2</sub>–N, m, 1P).

**MS**: (+) 993.5 [M+H]<sup>+</sup>; 1015.4 [M+Na]<sup>+</sup>.

Example 83: Synthesis of compound **58**

Into a glass vial (4 mL), compound **16**·3HCl·H<sub>2</sub>O (48.3 mg; 80.3 μmol) was weighted out. Consequently, aqueous HCl (1 M; 2 mL) and zinc powder (ca. 100 mg)

were added. The suspension was stirred at room temperature for 3 h. Subsequently, the mixture was filtered through syringe filter (Millipore; 0.22  $\mu\text{m}$ ). The filtrate was evaporated to dryness and the residue was dissolved in water (0.5 mL). Then, aqueous HCl (12 M; 1.5 mL) and *i*PrOH (2 mL) were added. The next day, the precipitate was centrifugated. The supernatant was decanted out the solid material was centrifugated with *i*PrOH (2 $\times$ 2 mL) and Et<sub>2</sub>O (1 $\times$ 2 mL). The product was dried in vacuum desiccator. The final product was obtained as a white material (26.6 mg; 54 %, 1 step, based on **16**·3HCl·H<sub>2</sub>O).

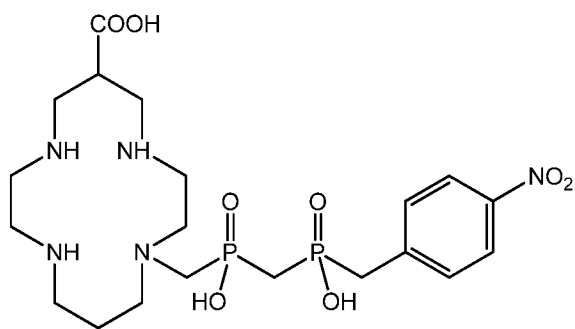
**NMR** (D<sub>2</sub>O): <sup>1</sup>H  $\delta$  1.82 (CH<sub>3</sub>, s, 3H); 1.97 (CH<sub>3</sub>, s, 3H); 2.15 (P—CH<sub>2</sub>—P, dd, 2H, <sup>2</sup>J<sub>HP</sub> = 18 Hz, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.64–3.34 (*cycle*, N—CH<sub>2</sub>—P m, 18H); 7.13 (PH, d, 1H, <sup>1</sup>J<sub>HP</sub> = 528 Hz); <sup>13</sup>C{<sup>1</sup>H}  $\delta$  23.9 (CH<sub>3</sub>, s); 25.3 (CH<sub>3</sub>, s); 34.9 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 80 Hz, <sup>1</sup>J<sub>CP</sub> = 76 Hz); 48.0 (*cycle*, s); 48.7 (*cycle*, s); 48.9 (*cycle*, s); 50.3 (*cycle*, s); 51.3 (*cycle*, s); 51.8 (*cycle*, s); 55.3 (*cycle*, s); 57.9 (C—NH<sub>2</sub>, s); 58.2 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 101 Hz); 58.4 (C—NH<sub>2</sub>, s); 59.3 (*cycle*, d, <sup>3</sup>J<sub>CP</sub> = 10 Hz); <sup>31</sup>P  $\delta$  19.8 (PH, dt, 1P, <sup>1</sup>J<sub>PH</sub> = 528 Hz, <sup>2</sup>J<sub>PH</sub> = 18 Hz); 33.7 (N—CH<sub>2</sub>—P, m, 1P).

**MS**: (–) 413.2 [M–H<sup>+</sup>]<sup>–</sup>. (+) 415.3 [M+H<sup>+</sup>]<sup>+</sup>; 437.3 [M+Na<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 1:1): R<sub>f</sub> = 0.4.

**EA** (C<sub>14</sub>H<sub>36</sub>N<sub>6</sub>O<sub>4</sub>P<sub>2</sub>·5HCl·H<sub>2</sub>O, M<sub>R</sub> = 614.7): C 27.4 (27.2); H 7.1 (7.2); N 13.7 (14.0).

20 Example 84: *Synthesis of compound 59*



Into a glass vial (4 mL), tetrahydrochloride of 1,4,8,11-tetraazacyclotetradecane-6-carboxylic acid (388 mg; 994  $\mu\text{mol}$ ; 3.0 equiv.), compound **B** (135 mg; 484  $\mu\text{mol}$ ; 1.5 equiv.) and paraformaldehyde (9.8 mg; 327  $\mu\text{mol}$ ; 1.0 equiv.) were weighted, and aqueous HCl (6 M; 1 mL) and trifluoroacetic acid (1 mL) were added. The mixture was stirred at 60 °C for three days. The solution was evaporated to dryness and the residue was co-evaporated with water several times. The crude product was purified by preparative HPLC (C8; H<sub>2</sub>O–0.1% TFA–MeCN). The fraction containing product

was lyophilized. The final product was obtained as a white material (49.7 mg; 24 %, 1 step, based on paraformaldehyde).

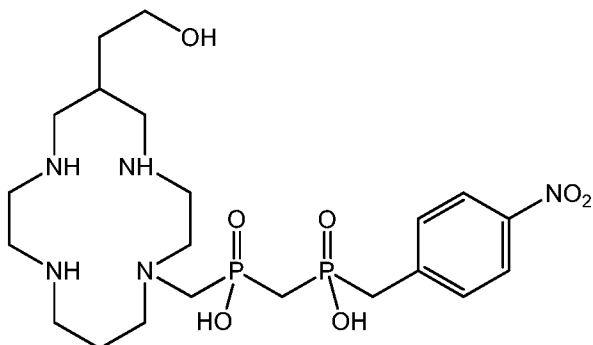
**NMR** ( $D_2O$ ):  $^1H$   $\delta$  1.77 (*cycle*, p, 2H,  $^3J_{HH} = 4$  Hz); 2.01 (P—CH<sub>2</sub>—P, t, 2H,  $^2J_{HP} = 17$  Hz); 2.33–2.99 (*cycle*, N—CH<sub>2</sub>—P, m, 19H); 3.25 (CH<sub>2</sub>—C—CH, d, 2H,  $^2J_{HP} = 16$  Hz); 7.61 (CH—C—CH<sub>2</sub>, d, 2H,  $^2J_{HH} = 8$  Hz); 8.27 (CH—C—N, d, 2H,  $^2J_{HH} = 8$  Hz);  $^{31}P\{^1H\}$   $\delta$  30.1 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P,  $^2J_{PP} = 11$  Hz); 32.8 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P,  $^2J_{PP} = 11$  Hz).

**MS**: (–) 515.9 [M+H<sup>+</sup>]<sup>+</sup>; 534.0 [M+H<sup>+</sup>]<sup>+</sup>. (+) 336.0 [M+H<sup>+</sup>]<sup>+</sup>.

**TLC** (SiO<sub>2</sub>, EtOH–conc. aq. NH<sub>4</sub>OH 1:1):  $R_f = 0.5$ .

**EA** (C<sub>20</sub>H<sub>35</sub>N<sub>6</sub>O<sub>8</sub>P<sub>2</sub>·0.5TFA·2H<sub>2</sub>O,  $M_R = 667.6$ ): C 40.1 (39.9); H 6.3 (6.6); N 11.1 (10.8).

Example 85: *Synthesis of compound 60*

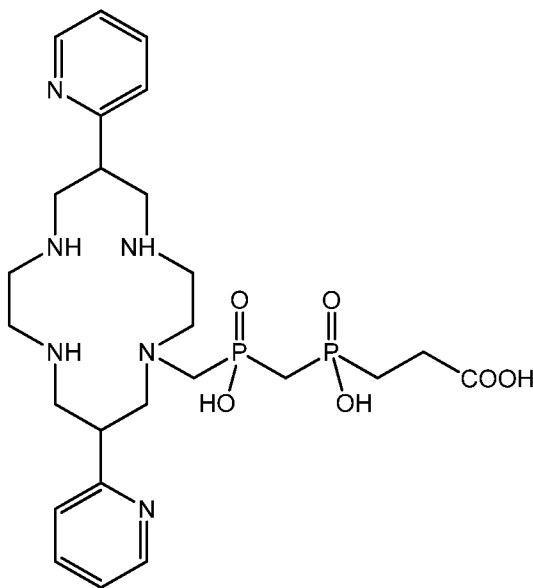


Into a glass vial (4 mL), 2-(1,4,8,11-tetraazacyclotetradecan-6-yl)ethanol hydrate (prepared according to N. Camus *et al.* *RSC Adv.* 2015, **5**, 85898–85910; 157 mg; 598  $\mu$ mol; 4.0 equiv.), compound **B** (97 mg; 348  $\mu$ mol; 2.3 equiv.) and paraformaldehyde (4.5 mg; 150  $\mu$ mol; 1.0 equiv.) were weighted, and aqueous HCl (12 M; 1 mL) was added. The mixture was stirred at 60 °C for two days. Consequently, water was added just to dissolve all solids. The solution was evaporated to dryness and the residue was co-evaporated with water several times. The crude product was purified by preparative HPLC (C8; H<sub>2</sub>O–0.1% TFA–MeCN). The fraction containing product was lyophilized. The final product was obtained as an off-yellow material (22.4 mg; 22 %, 1 step, based on paraformaldehyde).

**NMR** ( $D_2O$ ):  $^1H$   $\delta$  1.68–1.75 (*cycle*, CH<sub>2</sub>—CH<sub>2</sub>—O, bm, 4H); 2.01 (P—CH<sub>2</sub>—P, dd, 2H,  $^2J_{HP} = 17$  Hz,  $^2J_{HP} = 15$  Hz); 2.13 (*cycle*, m, 1H); 2.48–3.13 (*cycle*, N—CH<sub>2</sub>—P, m, 18H); 3.32 (CH<sub>2</sub>—C—CH, d, 2H,  $^2J_{HP} = 16$  Hz); 3.65 (CH<sub>2</sub>—OH, t,  $^3J_{HH} = 9$  Hz);

- 7.58 (CH—C—CH<sub>2</sub>, d, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz); 8.17 (CH—C—N, d, 2H, <sup>2</sup>J<sub>HH</sub> = 9 Hz);  
<sup>13</sup>C{<sup>1</sup>H} δ 24.9 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 31.3 (CH<sub>2</sub>—CH—CH<sub>2</sub>, s); 32.9 (CH<sub>2</sub>—CH<sub>2</sub>—  
 O, s); 34.2 (P—CH<sub>2</sub>—P, dd, <sup>1</sup>J<sub>CP</sub> = 82 Hz, <sup>1</sup>J<sub>CP</sub> = 79 Hz); 40.5 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> =  
 86 Hz); 44.1 (cycle, s); 45.0 (cycle, s); 45.6 (cycle, s); 45.9 (cycle, s); 46.2 (cycle, s);  
 5 47.1 (cycle, s); 55.1 (cycle, s); 55.2 (cycle, s); 58.3 (P—CH<sub>2</sub>—N, d, <sup>1</sup>J<sub>CP</sub> = 107 Hz);  
 62.4 (CH<sub>2</sub>—OH, s); 125.6 (CH—C—N, s); 129.9 (CH—C—CH<sub>2</sub>, d, <sup>3</sup>J<sub>CP</sub> = 5 Hz);  
 142.3 (CH—C—CH<sub>2</sub>, s); 147.1 (CH—C—N, s); <sup>31</sup>P{<sup>1</sup>H} δ 28.5 (CH<sub>2</sub>—P—CH<sub>2</sub>, d,  
 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz); 34.3 (CH<sub>2</sub>—P—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 10 Hz).  
 MS: (–) 534.2 [M–H]<sup>+</sup>. (+) 536.2 [M+H]<sup>+</sup>; 558.2 [M+Na]<sup>+</sup>; 574.2 [M+K]<sup>+</sup>.  
 10 TLC (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3): R<sub>f</sub> = 0.6.  
 EA (C<sub>21</sub>H<sub>39</sub>N<sub>5</sub>O<sub>7</sub>P<sub>2</sub>·TFA·H<sub>2</sub>O, M<sub>R</sub> = 667.6): C 41.4 (41.7); H 6.3 (5.9); N 10.5 (10.3).

Example 86: *Synthesis of compound 61*



- 15 Into a glass vial (20 mL), tetrahydroperchlorate 6,13-di(2-pyridyl)-1,4,8,11-  
 tetrazacyclotetradecane dihydrate (synthesized according to P. Comba et al. *Inorg. Chem.*  
 2001, **40**, 2335–2345; 1.11 g; 1.40 mmol; 3.3 equiv.), compound **D** (128 mg; 592 μmol;  
 1.4 equiv.) and paraformaldehyde (12.7 mg; 423 μmol; 1.0 equiv.) were weighted, and  
 aqueous HCl (12 M; 5 mL) was added. The mixture was stirred at 50 °C for two days. The  
 20 reaction mixture was evaporated to dryness and the residue was co-evaporated with water  
 several times. The crude product was purified by ion exchange chromatography  
 (DOWEX 50; H<sup>+</sup>-form, H<sub>2</sub>O → 10 % aq. pyridine). The pyridine fraction containing  
 product was evaporated to dryness and the the residue was co-evaporated with water

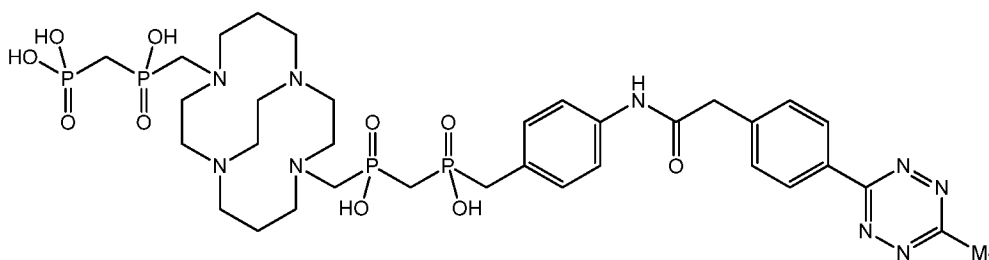
several times. The residue was then dried by vacuum pump obtained and further drying was accomplished with desiccator over P<sub>2</sub>O<sub>5</sub>. The final product was obtained as an off-yellow material (184 mg; 66 %, 1 step, based on paraformaldehyde).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.98 (P—CH<sub>2</sub>—C, m, 2H); 2.08 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 17 Hz);  
 5 2.41 (CH<sub>2</sub>—CO, m, 2H); 2.63–2.89 (*cycle*, N—CH<sub>2</sub>—P, CH<sub>2</sub>—CH—CH<sub>2</sub>, m, 20H);  
 7.48 (*arom.*, m, 4H); 7.66 (*arom.*, dd, 2H, <sup>3</sup>J<sub>HH</sub> = 8 Hz, <sup>3</sup>J<sub>HH</sub> = 6 Hz); 8.38 (*arom.*, dm,  
 2H, <sup>3</sup>J<sub>HH</sub> = 6 Hz); <sup>13</sup>C{<sup>1</sup>H} δ 29.5 (P—CH<sub>2</sub>—C, d, <sup>1</sup>J<sub>CP</sub> = 95 Hz); 31.6 (CH<sub>2</sub>—CO, s);  
 34.0 (P—CH<sub>2</sub>—P, t, <sup>1</sup>J<sub>CP</sub> = 81 Hz); 40.3 (CH<sub>2</sub>—CH—CH<sub>2</sub>, s); 41.5 (CH<sub>2</sub>—CH—CH<sub>2</sub>,  
 s); 44.0 (*cycle*, s); 46.5 (*cycle*, s); 46.9 (*cycle*, s); 47.2 (*cycle*, s); 47.8 (*cycle*, s); 50.3  
 10 (*cycle*, s); 54.5 (*cycle*, s); 55.1 (N—CH<sub>2</sub>—P, d, <sup>1</sup>J<sub>CP</sub> = 109 Hz); 55.8 (*cycle*, s); 124.9  
 (*arom.*, s); 125.3 (*arom.*, s); 127.4 (*arom.*, s); 131.6 (*arom.*, s); 162.0 (*arom.*, s);  
 182.6 (CO, d, <sup>3</sup>J<sub>CP</sub> = 17 Hz); <sup>31</sup>P{<sup>1</sup>H} δ 32.2 (P—CH<sub>2</sub>—N, d, 1P, <sup>2</sup>J<sub>PP</sub> = 11 Hz); 37.8  
 (P—CH<sub>2</sub>—CH<sub>2</sub>, d, 1P, <sup>2</sup>J<sub>PP</sub> = 11 Hz).

**MS**: (–) 563.1 [M–H<sup>+</sup>]<sup>–</sup>; 581.0 [M–H<sup>+</sup>]<sup>–</sup>. (+) 583.2 [M+H<sup>+</sup>]<sup>+</sup>; 605.1 [M+Na<sup>+</sup>]<sup>+</sup>; 621.1  
 15 [M+K<sup>+</sup>]<sup>+</sup>.

**EA** (C<sub>25</sub>H<sub>40</sub>N<sub>6</sub>O<sub>6</sub>P<sub>2</sub>·4H<sub>2</sub>O, M<sub>R</sub> = 654.6): C 45.9 (46.1); H 7.4 (7.3); N 12.8 (13.0).

Example 87: *Synthesis of conjugate of compound 38 with tetrazine*



20 Into a glass vial (4 mL), compound 38·4H<sub>2</sub>O (33.3 mg; 45.5 μmol; 1.0 equiv.) and  
 NHS ester of 2-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)acetic acid (16.4 mg; 50.1  
 μmol; 1.1 equiv.) were weighted, and aqueous buffer MES/NaOH (1.0 M; pH 6.2, 1.5  
 mL) and MeCN (1.5 mL) were added. The mixture was stirred at room temperature for  
 two days. Consequently, water was then directly purified by preparative HPLC (C8;  
 25 H<sub>2</sub>O–0.1% TFA–MeCN). The fraction containing product was lyophilized. The final  
 product was obtained as a pink material (19.2 mg; 43 %, 1 step, based on 38·4H<sub>2</sub>O).

**NMR** (D<sub>2</sub>O): <sup>1</sup>H δ 1.97 (*cycle*, bm, 2H); 2.26 (*cycle*, bm, 2H); 2.31 (P—CH<sub>2</sub>—P, t,  
 2H, <sup>2</sup>J<sub>HP</sub> = 16 Hz); 2.36 (P—CH<sub>2</sub>—P, t, 2H, <sup>2</sup>J<sub>HP</sub> = 19 Hz); 2.71–3.86 (*cycle*, N—  
 CH<sub>2</sub>—P, P—CH<sub>2</sub>—C, CH<sub>3</sub>, bm, 29H); 3.93 (CH<sub>2</sub>—CO, s, 2H); 7.38 (CH, d, 2H, <sup>3</sup>J<sub>HH</sub>

- = 8 Hz); 7.46 (CH, d, 2H,  $^3J_{\text{HH}} = 8$  Hz); 7.67 (CH, d, 2H,  $^3J_{\text{HH}} = 8$  Hz); 8.43 (CH, d, 2H,  $^3J_{\text{HH}} = 8$  Hz);  $^{13}\text{C}\{^1\text{H}\}$  20.4 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, bm); 20.6 (CH<sub>2</sub>—CH<sub>2</sub>—CH<sub>2</sub>, s); 20.7 (CH<sub>3</sub>, s); 32.8 (2 × P—CH<sub>2</sub>—P, m); 38.6 (P—CH<sub>2</sub>—C, d,  $^1J_{\text{CP}} = 91$  Hz); 43.6 (CH<sub>2</sub>—CO, s); 47.4–58.7 (12 × N—CH<sub>2</sub>, bm); 126.7 (CH—C—N, s); 130.1 (CH—C—N, s); 131.8 (CH—C—CH<sub>2</sub>, s); 132.2 (CH—C—CH<sub>2</sub>, d,  $^2J_{\text{CP}} = 8$  Hz); 164.7 (N—C—N, s); 167.9 (N—C—N, s); 173.2 (CO, s);  $^{31}\text{P}\{^1\text{H}\}$   $\delta$  16.0 (HO—P—OH, m, 1P); 22.5 (P—CH<sub>2</sub>—N, bm, 1P); 23.5 (P—CH<sub>2</sub>—N, bm, 1P); 38.8 (P—CH<sub>2</sub>—C, m, 1P).  
 MS: (–) 870.3 [M–H<sup>+</sup>]<sup>–</sup>. (+) 872.5 [M+H<sup>+</sup>]<sup>+</sup>; 894.5 [M+Na<sup>+</sup>]<sup>+</sup>; 910.5 [M+K<sup>+</sup>]<sup>+</sup>.  
 TLC (SiO<sub>2</sub>, *i*PrOH–conc. aq. NH<sub>4</sub>OH–H<sub>2</sub>O 7:3:3):  $R_f = 0.4$ .  
 EA (C<sub>34</sub>H<sub>53</sub>N<sub>9</sub>O<sub>10</sub>P<sub>4</sub>·TFA,  $M_R = 985.8$ ): C 43.9 (43.8); H 5.5 (5.3); N 12.8 (13.1).

Example 88: *Conjugation of compound 15 to copolymer containing poly(acrylamide)*

- Block copolymer of poly[*N*-(2-hydroxypropyl)methakrylamide and poly-L-lysine (ten L-Lys residues) was obtained according to K. Tappertzhofen et al. *Macromolec. Biosci.* 2015, **15**, 1159–1173 (average  $M_w$  14 kDa, one poly-L-Lys chain per the copolymer). Compound **15** (93 mg; 135  $\mu\text{mol}$ ; 1.5 equiv. per an amino group) and *N*-hydroxysuccinimide (NHS; 15.5 mg; 135  $\mu\text{mol}$ ; 1.5 equiv.) were dissolved in aqueous MES/NaOH buffer (pH 5.5; 0.5 M; 2 mL). Subsequently, a DMSO/H<sub>2</sub>O 1:1 (3.0 mL) solution containing 140 mg (cca 90  $\mu\text{mol}$  of amino groups; 1 equiv.) of the polymer was added. To this solution, solid EDC·HCl (26 mg; 135  $\mu\text{mol}$ ; 1.5 equiv.) was added and the mixture was stirred at room temperature for 2 days. Then, the reaction mixture was concentrated to 2 mL and the solution was filtered through a syringe filter (Millipore; 0.22  $\mu\text{m}$ ) into ultrafiltration cell. Ultrafiltration (pressure 3 atm) on 3-kDa membrane was carried out three times from 50 mL to 10 mL of the retentate and it was followed by continuous ultrafiltration (pressure 4 atm) until 1000 mL of eluate was obtained. The retentate was lyophilized to give a white solid (175 mg). An average ligand number bound to the polymer was determined (<sup>1</sup>H NMR spectrum) to be ca. 8.0

Example 89 *Conjugate of compound 39 and poly(acrylamide) with free amino groups*

- Poly[*N*-(2-hydroxypropyl)methakrylamide with free amino groups was prepared the polymer active ester consecutive reactions, first with NH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>NH-C(O)CH<sub>2</sub>NHBoc and later with 2-hydroxypropylaminem, which were followed by Boc protecting group removal, according to L. Nuhn et al. *Angew. Chem. Int. Ed.* 2013, **52**,

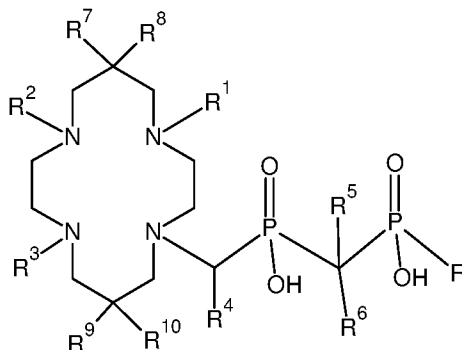
10652–10656 (average  $M_w$  16 kDa, ca. 22 free amino groups per molecule). A buffer MOPS/NaOH (5 mL, pH 7.5, 0.5 M) solution was added to the polymer with free glycine amino groups (105 mg, ca. 140  $\mu$ mol amino groups) and the compound **39** (111 mg; 140  $\mu$ mol, equivalent amount to a number of the amine groups) and the solution was stirred at  
5 room temperature for 12 h.. The solution was filtered through a syringe filter (Millipore; 0.22  $\mu$ m) into ultrafiltration cell. Ultrafiltration (pressure 3 atm) on 3-kDa membrane was carried out three times from 50 mL to 10 mL of the retentate and it was followed by continuous ultrafiltration (pressure 4 atm) until 1000 mL of eluate was obtained. The retentate was lyophilized to give a white solid (185 mg). An average ligand number bound  
10 to the polymer was determined ( $^1\text{H}$  NMR spectrum) to be 19.6

Example 90 *Conjugate of compound 57 and poly(acrylamide) through thiol coupling*

Poly[N-(2-hydroxypropyl)methakrylamide with free maleimide groups was prepared the polymer active ester by consecutive reactions, first with  
15 aminohexamethylene-maleimide and later with 2-hydroxypropylaminem according to L. Nuhn et al. *Angew. Chem. Int. Ed.* 2013, **52**, 10652–10656 (average  $M_w$  18 kDa, ca. 19 free maleimide groups per polymer). Polymer (115 mg, ca. 120  $\mu$ mol maleimide groups) was dissolved in aqueous buffer MOPS/NaOH (5 mL; 0.5 M, pH 7.5) and compound **57** (86 mg; 60  $\mu$ mol) was added, i.e. equivalent amount of –SH groups per a number of  
20 reactive groups per polymer was used. The solution was bubbled with argon (10 min). Free –SH group was generated *in-situ* after addition of tris(2-karboxyethyl)phosphine hydrochloride (TCEP·HCl, 17 mg, 60  $\mu$ mol) in the argon flow and the mixture was stirred with no air access for 12 h. In the argon flow, compound **57** (43 mg; 30  $\mu$ mol, i.e. ca. 0.5 molar equivalent with regard to a number of reactive groups on the polymer) dissolved in  
25 aqueous buffer (2 mL; 0.1 M, pH 7.5) was added, it was followed by addition of TCEP·HCl (11 mg, cca 35  $\mu$ mol) and the mixture was stirred with no air access for 12 h. The solution was filtered through a syringe filter (Millipore; 0.22  $\mu$ m) into ultrafiltration cell. Ultrafiltration (pressure 3 atm) on 3-kDa membrane was carried out three times from 50 mL to 10 mL of the retentate and it was followed by continuous ultrafiltration (pressure  
30 4 atm) until 900 mL of eluate was obtained. The retentate was lyophilized to give a white solid (185 mg). An average ligand number bound to the polymer was determined ( $^1\text{H}$  NMR spectrum) to be 16.5

## CLAIMS

1. Cyclam based compounds of the general formula (I)

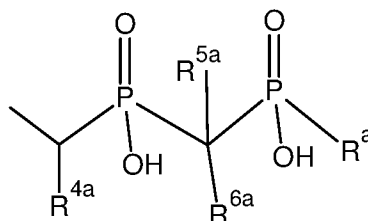


5

(I)

wherein

R, R<sup>a</sup>, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> are independently selected from the group comprising H, OH, (C1 to C6)alkyl, which may be linear or branched, (C3 to C6)cycloalkyl, benzyl, and/or R<sup>1</sup> and/or R<sup>2</sup> and/or R<sup>3</sup> is a bis-phosphorus acid of the general formula (II)



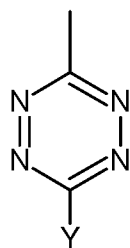
10

(II)

and/or R<sup>1</sup> and R<sup>3</sup> together form (C2 to C3)alkylene, which may be substituted with one or more linear or branched (C1 to C6)alkyls,

and/or R, R<sup>a</sup> are independently selected from phenyl and (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O,

15 wherein the (C1 to C6)alkyl, (C3 to C6)cycloalkyl, phenyl, benzyl and (C5 to C6) heterocycle, containing at least one heteroatom of N, S, O, may be independently substituted with one or more groups selected from COOH; NH<sub>2</sub>; NO<sub>2</sub>, NX<sub>2</sub>; C(O)NX<sub>2</sub>; NHX; C(O)NHX; OH; SH; -NCS; tetrazine of  
20 the general formula (III);



(III)

(C7 to C8)cycloalkenyl in *trans* configuration, which may eventually be further substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups chosen from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cycloazaocynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z;

and wherein R<sup>4</sup>, R<sup>4a</sup>, R<sup>5</sup> and R<sup>5a</sup> are independently H, (C1 to C6)alkyl, which may be linear or branched, (C3 to C6)cycloalkyl, benzyl;

wherein (C1 to C6)alkyl, (C3 to C6)cycloalkyl and benzyl may be independently substituted with one or more groups selected from COOH; NH<sub>2</sub>; NO<sub>2</sub>, NX<sub>2</sub>; C(O)NX<sub>2</sub>; NHX; C(O)NHX; OH; SH; -NCS; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans* configuration, which may eventually be further substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cycloazaocynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z;

and wherein R<sup>6</sup> and R<sup>6a</sup> is H;

and wherein R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup> and R<sup>10</sup> are independently selected from the group comprising H, (C1 to C6)alkyl, (C3 to C6)cycloalkyl, benzyl, phenyl, NO<sub>2</sub>, COOH, (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O, and/or altogether R<sup>7</sup> and R<sup>8</sup> contain (C2 to C3)alkylene or vinylene and/or altogether R<sup>9</sup> and R<sup>10</sup> contain (C2 to C3)alkylene or vinylene,

wherein (C1 to C6)alkyl, (C3 to C6)cycloalkyl, phenyl, benzyl, (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O, (C2 to C3)alkylene and vinylene may be independently substituted with a group or groups selected from COOH; NH<sub>2</sub>; NO<sub>2</sub>; NX<sub>2</sub>; C(O)NX<sub>2</sub>; SH; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans* configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cycloazaoctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z,

wherein Y is H, (C1 to C6)alkyl, -CH<sub>2</sub>COOH, (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O, or heteroaryl, containing at least one heteroatom of N, S, O, preferably Y is an atom of hydrogen, methyl or pyridyl;

wherein Z is H; OH; (C1 to C6)alkyl, which may be linear or branched; (C1 to C6)hydroxyalkyl; (C1 to C6)alkoxyl; phenyl; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; or benzyl, wherein phenyl, (C5 to C6)heterocycle or benzyl may eventually be substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub> and SH, preferably the substituent is in *para* position;

and wherein X is independently H; (C1 to C6)alkyl, which may be linear or branched; (C3 to C6)cycloalkyl; phenyl and/or benzyl;

while for X, Y and Z applies, that (C1 to C6)alkyl, (C3 to C6)cycloalkyl, phenyl and/or benzyl may be independently substituted with one or more groups selected from COOH; NH<sub>2</sub>; NO<sub>2</sub>; NX<sub>2</sub>; C(O)NX<sub>2</sub>; NHX; C(O)NHX;

SH; tetrazine of the general formula **(III)**; (C7 to C8)cycloalkenyl in *trans* configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; -N<sub>3</sub>; (C2 to C6)alkynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; cycloazaoctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, -NCS, -NCO, N<sub>3</sub> and SH; maleimide; phenyl; benzyl and -P(O)(OH)Z;

wherein at least one of the groups R, R<sup>a</sup>, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>4a</sup>, R<sup>5</sup>, R<sup>5a</sup>, R<sup>6</sup>, R<sup>6a</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup> or R<sup>10</sup> contains at least one of the groups -COOH; NH<sub>2</sub>; NO<sub>2</sub>; NX<sub>2</sub>; NHX; C(O)NHX; N<sub>3</sub>; SH; -NCS; tetrazine of the general formula **(III)**; (C7 to C8)cycloalkenyl in *trans* configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; -N<sub>3</sub>; (C2 to C6)alkynyl; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; cycloazaoctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; maleimide; phenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; benzyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; (C1 to C6)alkyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, N<sub>3</sub> and SH; and -P(O)(OH)Z.

2. The cyclam based compounds according to claim 1, **characterized in that** R<sup>1</sup> and R<sup>3</sup> together form (C2 to C3)alkylene, which may be substituted with (C1 to C6)alkyl, which may be linear or branched.

3. The cyclam based compounds according to claim 1, **characterized in that** R, R<sup>a</sup>, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>4a</sup>, R<sup>5</sup>, R<sup>5a</sup>, R<sup>6</sup> and R<sup>6a</sup> are independently selected from the group comprising

H; (C1 to C6)alkyl, which may be linear or branched; (C3 to C6) cycloalkyl; benzyl; phenyl; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; and/or R<sup>1</sup> and/or R<sup>2</sup> and/or R<sup>3</sup> is the bis-phosphorus acid of the general formula (II), preferably at least one of the groups R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> is the bis-phosphorus acid of the general formula (II), most preferably R<sup>2</sup> is the bis-phosphorus acid of the general formula (II).

4. The cyclam based compounds according to claim 1, **characterized in that** R<sup>2</sup> is selected from the group comprising H; (C1 to C6)alkyl, which may be linear or branched; (C3 to C6)cycloalkyl; benzyl; phenyl; (C5 to C6)heterocycle, containing at least one heteroatom of N, S, O; and bis-phosphorus acid of the general formula (II).

5. The cyclam based compounds according to claim 1, **characterized in that** they are selected from the group comprising compounds of the general formula (I), whose the substituents are represented in the following combinations:

Compound number	R	R <sup>1</sup>	R <sup>3</sup>	R <sup>2</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	R <sup>7</sup>	R <sup>8</sup>	R <sup>9</sup>	R <sup>10</sup>
1	H	H	H	H	CH <sub>3</sub>	H	H	H	H	H	H
2	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	H	H	H
3	H	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	H	H	H	H	H
4	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	H	H	H	H
5	OH	benzyl	benzyl	H	H	H	H	H	H	H	H
7	OH	H	benzyl	benzyl	H	H	H	H	H	H	H
8	<i>p</i> -nitrobenzyl	H	H	H	H	H	H	H	H	H	H
9	<i>p</i> -aminobenzyl	H	H	H	H	H	H	H	H	H	H
10	<i>p</i> -SCN-benzyl	H	H	H	H	H	H	H	H	H	H
11	<i>p</i> -azidobenzyl	H	H	H	H	H	H	H	H	H	H
12	-CH <sub>2</sub> N(Bz) <sub>2</sub>	H	H	H	H	H	H	H	H	H	H
13	-CH <sub>2</sub> NH <sub>2</sub>	H	H	H	H	H	H	H	H	H	H
14	-(CH <sub>2</sub> ) <sub>2</sub> COOH	H	H	H	H	H	H	H	H	H	H
15	H	H	H	H	H	-(CH <sub>2</sub> ) <sub>4</sub> COOH	H	H	H	H	H



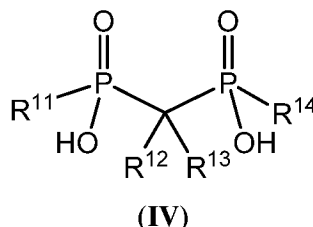




6. A conjugate of the cyclam-based compound according to any one of the preceding claims and of at least one conjugation group, which is covalently bound to the cyclam based compound, and which is selected from the group containing OH, -COOH; NH<sub>2</sub>; NO<sub>2</sub>, NX<sub>2</sub>, NHX; C(O)NHX; N<sub>3</sub>; SH; -NCS; tetrazine of the general formula (III); (C7 to C8)cycloalkenyl in *trans* configuration, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX; C(O)NHX; N<sub>3</sub> and SH; norbornenyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX; C(O)NHX; N<sub>3</sub> and SH; -N<sub>3</sub>; (C2 to C6)alkynyl; cyclooctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX; C(O)NHX; N<sub>3</sub> and SH; cyclozaaoctynyl, eventually substituted with one or more groups selected from COOH, NH<sub>2</sub>, NO<sub>2</sub>, NX<sub>2</sub>, -NCS, -NCO, NHX, C(O)NHX, (C1 to C6)alkyl, (C6)aryl, N<sub>3</sub> and SH; maleimide; and -P(O)(OH)Z; wherein the conjugate may further contain a spacer between the cyclam-based compound and the conjugation group and/or between two conjugation groups, the spacer selected from the group containing (C1 to C6)*n*-alkyl, eventually substituted with C=O and/or -NH- group; phenylene; amino-acids chains with the length of 1 to 5 amino-acids; polyethylene glycols of 1 to 10 monomeric units.
7. Coordination compounds of the cyclam based compounds of the general formula (I) according to any one of the claims 1 to 5 or of the conjugates according to the claim 6, with metal cations selected from the group consisting of Cu<sup>2+</sup>, Bi<sup>3+</sup>, lanthanide(III) cations, Sc<sup>3+</sup>, Y<sup>3+</sup>, Pb<sup>2+</sup>, Zr<sup>4+</sup>, Ac<sup>3+</sup>, Mn<sup>2+</sup> and Mn<sup>3+</sup>, preferably lanthanide(III) cations, Y<sup>3+</sup> a Cu<sup>2+</sup>, more preferably Lu<sup>3+</sup>, Y<sup>3+</sup> and Cu<sup>2+</sup>.
8. A targeting conjugate, which contains the cyclam based compound of the general formula (I) according to any one of the claims 1 to 5 and/or the conjugate according to the claim 6 and/or the coordination compound according to the claim 7; and a targeting vector, selected from the group comprising bis(phosphonates); groups capable of fluorescence, preferably fluoresceines, rhodamines or the boron-dipyrromethene type substances; oligopeptides of 1 to 15 aminoacids; antibodies or fragments thereof, preferably the Fab fragments; folic acid; biotin; compounds targeting to PSMA receptor, preferably urea derivatives or organo-phosphorous compounds; cyclodextrins;

dendrimers; hydrophilic polymers on the basis of derivatives of acrylic acid, especially hydroxoalkylamides of acrylic acid.

9. A method of preparation of cyclam based compounds of the general formula (I)  
5 according to any one of the claims 1 to 5, wherein an intermediate product of the general formula (IV),



wherein R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup> and R<sup>14</sup> are in the following combinations:

Intermediate	R <sup>11</sup>	R <sup>12</sup>	R <sup>13</sup>	R <sup>14</sup>
A	H	H	H	OH
B	H	H	H	<i>p</i> -nitrobenzyl
C	H	H	H	-CH <sub>2</sub> N(benzyl) <sub>2</sub>
D	H	H	H	-(CH <sub>2</sub> ) <sub>2</sub> COOH
E	H	-(CH <sub>2</sub> ) <sub>4</sub> COOH	H	H
F	OH	-(CH <sub>2</sub> ) <sub>3</sub> NH(4-dibenzylcyclooctynamidobutoxyl)	OH	OH

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or a compound selected from the group containing methylene-bis(phosphinic acid), phosphorous acid, hydroxymethylphosphinic acid, reacts with a cyclam derivative and an aldehyde in water solution of an acid of a concentration within the range of from 10 to 40 % (weight), preferably from 18 to 36 % (weight), at the temperature in the range of  
15 from 40 °C to 80 °C for the period of time of at least 12 hours;

wherein the cyclam derivative is 1,4,8,11-tetraazacyclotetradecane and derivatives thereof and 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane and derivatives thereof, preferably the cyclam derivative is selected from the group containing 1,4,8,11-tetraazacyclotetradecane, 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane, 1,4,8-trimethyl-  
20 1,4,8,11-tetraazacyclotetradecane, 1,8-dimethyl-1,4,8,11-tetraazacyclotetradecane, 1,8-dibenzyl-1,4,8,11-tetraazacyclotetradecane, 1,4-dibenzyl-1,4,8,11-tetraazacyclotetradecane, 6,13-dimethyl-6,13-dinitro-1,4,8,11-tetraazacyclotetradecane;

the aldehyde is selected from the group containing acetaldehyde, formaldehyde and para-formaldehyde, and the acid is preferably HCl and/or CF<sub>3</sub>COOH; and wherein the reaction may be preceded by the step of preparation of the intermediate product.

5 10. The method of preparation of the cyclam based compounds according to the claim 9, **characterized in that** the resulting product further undergoes a reduction reaction.

11. The method of preparation of the cyclam based compounds according to the claim 9, **characterized in that** the resulting product further undergoes an oxidation reaction.

10

12. The method of preparation of the cyclam based compounds according to the claim 9, **characterized in that** the resulting product further undergoes a reaction with an aldehyde; and eventually with an intermediate product of the general formula (IV), or with a compound, selected from the group containing methylene-bis(phosphinic acid), phosphorous acid, alkylphosphinic acid or arylphosphinic acid; in water solution of an acid.

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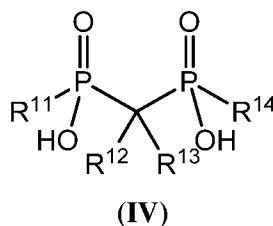
13. The method of preparation of the cyclam based compounds according to the claim 10, **characterized in that** the reduction step is followed by a step when the product of the reduction undergoes a reaction with CCl<sub>4</sub> or COCl<sub>2</sub>.

20

14. The method of preparation of the cyclam based compounds according to the claim 13, **characterized in that** the reduction step is followed by a step when the resulting product of the reduction undergoes a reaction with NaN<sub>3</sub>.

25

15. An intermediate product of the general formula (IV) for the preparation of cyclam based compounds,



30 wherein R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup> and R<sup>14</sup> are in the following combinations:

Intermediate	R <sup>11</sup>	R <sup>12</sup>	R <sup>13</sup>	R <sup>14</sup>

B	H	H	H	<i>p</i> -nitrobenzyl
C	H	H	H	-CH <sub>2</sub> N(benzyl) <sub>2</sub>
D	H	H	H	-(CH <sub>2</sub> ) <sub>2</sub> COOH
E	H	-(CH <sub>2</sub> ) <sub>4</sub> COOH	H	H
F	OH	-(CH <sub>2</sub> ) <sub>3</sub> NH(4-dibenzylcyclooctynamidobutoxyl)	OH	OH

16. A pharmaceutical preparation, **characterized in that** it contains at least one cyclam based compound according to any one of the claims 1 to 5 and/or at least one conjugate according to the claim 6 and/or at least one coordination compound according to the claim 7 and/or at least one targeting conjugate according to the claim 8, and a pharmaceutically acceptable substance.

17. The cyclam based compound according to any one of the claims 1 to 5 and/or at least one conjugate according to claim 6 and/or at least one coordination compound according to the claim 7 and/or at least one targeting conjugate according to the claim 8 and/or the pharmaceutical preparation according to the claim 16, for use as a medicament, preferably in the treatment of tumor diseases, inflammations, and/or atherosclerosis.

18. The cyclam based compound according to any one of the claims 1 to 5 and/or at least one conjugate according to claim 6 and/or at least one coordination compound according to the claim 7 and/or at least one targeting conjugate according to the claim 8 and/or the pharmaceutical preparation according to the claim 16, for use as a contrast agent in medical diagnostics, preferably in the diagnostics of tumor diseases, inflammations, and/or atherosclerosis.

19. Use of cyclam based compounds according to any one of the claims 1 to 5 and/or at least one conjugate according to claim 6 and/or at least one coordination compound according to the claim 7 and/or at least one targeting conjugate according to the claim 8 and/or the pharmaceutical preparation according to the claim 16, in radiochemistry as markers or precursors of markers when labelling with the use of radioisotopes of copper, lutetium, bismuth and yttrium.

20. Use of cyclam based compounds according to any one of the claims 1 to 5 and/or at least one conjugate according to claim 6 and/or at least one coordination compound according to the claim 7 and/or at least one targeting conjugate according to the claim 8,
- 5 for the complexation and/or purification of copper radioisotopes.

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/CZ2016/050043

A. CLASSIFICATION OF SUBJECT MATTER INV. C07F9/30 C07F9/38 A61K49/06 A61B5/00 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) C07F A61K A61B		
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,P	TOMÁS DAVID ET AL: "Cyclam Derivatives with a Bis(phosphinate) or a Phosphinato-Phosphonate Pendant Arm: Ligands for Fast and Efficient Copper(II) Complexation for Nuclear Medical Applications", INORGANIC CHEMISTRY, vol. 54, no. 24, 21 December 2015 (2015-12-21), pages 11751-11766, XP055335693, EASTON, US ISSN: 0020-1669, DOI: 10.1021/acs.inorgchem.5b01791 the whole document  ----- -/--	1-20
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> 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  18 January 2017		Date of mailing of the international search report  26/01/2017
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer  Johnson, Claire

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/CZ2016/050043

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012/150302 A1 (UNIV MUENCHEN TECH [DE]; NOTNI JOHANNES [DE]; WESTER HANS-JUERGEN [DE]) 8 November 2012 (2012-11-08) claims 1,3,7,13 -----	1-20
A	MONIKA PAÚROVÁ ET AL: "Bifunctional Cyclam-Based Ligands with Phosphorus Acid Pendant Moieties for Radiocopper Separation: Thermodynamic and Kinetic Studies", CHEMISTRY - A EUROPEAN JOURNAL., vol. 21, no. 12, 3 February 2015 (2015-02-03), pages 4671-4687, XP055335710, WEINHEIM, DE ISSN: 0947-6539, DOI: 10.1002/chem.201405777 the whole document -----	1-20

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/CZ2016/050043

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012150302	A1	NONE	08-11-2012