Studies on the Synthesis and Biological Properties of Non-Carrier-Added [^{125}I and ^{131}I]-Labeled Arylalkylidenebisphosphonates: Potent Bone-Seekers for Diagnosis and Therapy of Malignant Osseous Lesions

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Received December 9, 2002

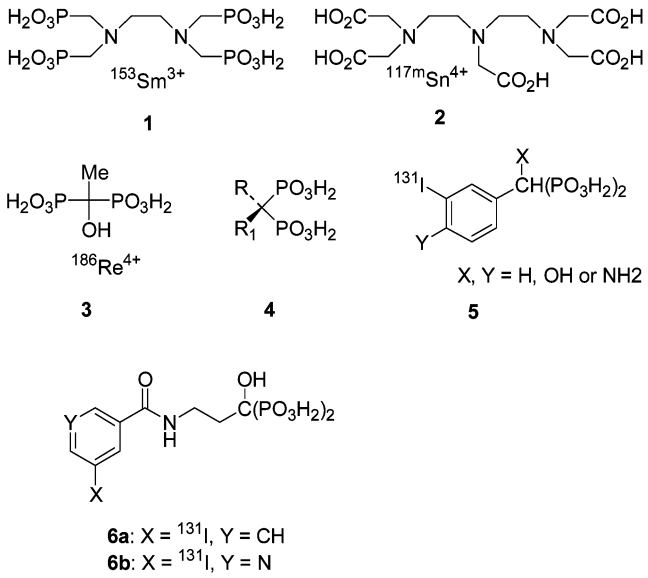
Arylalkylidenebisphosphonates labeled with nca [^{125}I or ^{131}I] have been synthesized and their biological function investigated. The label was attached to the aromatic group in high yield and under mild conditions by means of iododesilylation. The bone affinities of the radioactive compounds were investigated in normal Balb/C mice. The compound 1-hydroxy(*m*-iodo[^{125,131}I]-phenylethylidene)-1,1-bisphosphonate was found to possess superior bone affinity compared to others, and its in vivo deiodination was insignificant. The uptake in femur 24h after injection was 850 ± 265% and 986 ± 118% of injected dose per gram tissue times gram body weight in mice and rats, respectively. The therapeutic potential of the compound was investigated in two tumor models in athymic (nude) rats, one model for mixed lytic/sclerotic metastatic bone-lesions originating from breast cancer and the other model simulating osseous osteosarcoma. The effects in these models compare favorably to those observed for established treatment modalities. The experiments demonstrate that radioiodinated bisphosphonates may have a potential for diagnosis and therapy of malignant osseous lesions.

Introduction

The treatment of skeletal metastases is a serious problem in practical clinical oncology as the majority of patients with advanced carcinomas of the breast, prostate, or lung suffer from the condition.[1] The prognosis of patients with bone metastases varies from only a few months to some years, and the number of patients achieving complete cure is negligible.[2] At present, the current clinical practice for treatment includes external radiotherapy, hormone therapy, and chemotherapy. Severe side effects often limit the use of these methods. Consequently, there is a strong need for improved therapeutic methods to slow down tumor progression and alleviate pain. Bone-seeking radiopharmaceuticals are an attractive alternative to external beam irradiation,[3] because these compounds tend to concentrate with preference to areas of tumor growth.[4] As a result, targeted radiotherapy may spare healthy tissue from damage and thereby provide an opportunity to increase the radiation dose to tumor.

So far, the US Food and Drug Administration has approved only three bone-seeking radiopharmaceuticals, [^{32}P] sodium orthophosphate, [^{89}Sr] strontium chloride, and [^{153}Sm] samarium EDTMP (**1**).[5] For many years [^{32}P] sodium orthophosphate was the most widely used compound, but because of severe side effects[5] it has been replaced by [^{89}Sr] strontium chloride (Metastron). Sr^{2+} is preferentially taken up at sites of new bone formation, and it provides pain relief in up to 80% of the patients,[6,7] whereas [^{153}Sm] samarium EDTMP has shown potential for treatment of osseous osteosarcoma.[8,9] The limiting factor in the use of either [^{32}P] sodium orthophosphate or [^{89}Sr] strontium chloride is bone marrow depression, probably caused by penetration into the bone marrow space of the highly energetic β-radiation. Looking for more favorable decay modes, the most successful approach has been the use of metal complexes of radionuclides decaying by emission of medium- to low-energy β-particles or conversion electrons. [^{153}Sm] samarium EDTMP(**1**), [^{117m}Sn] tin DTPA (**2**), and [^{186}Re] rhenium HEDP(**3**) are examples of this category.[10−12] Moreover, bisphosphonates incorporating ^{131}I such as compounds **5** and **6** (Chart 1) have also shown considerable promise.

Chart 1



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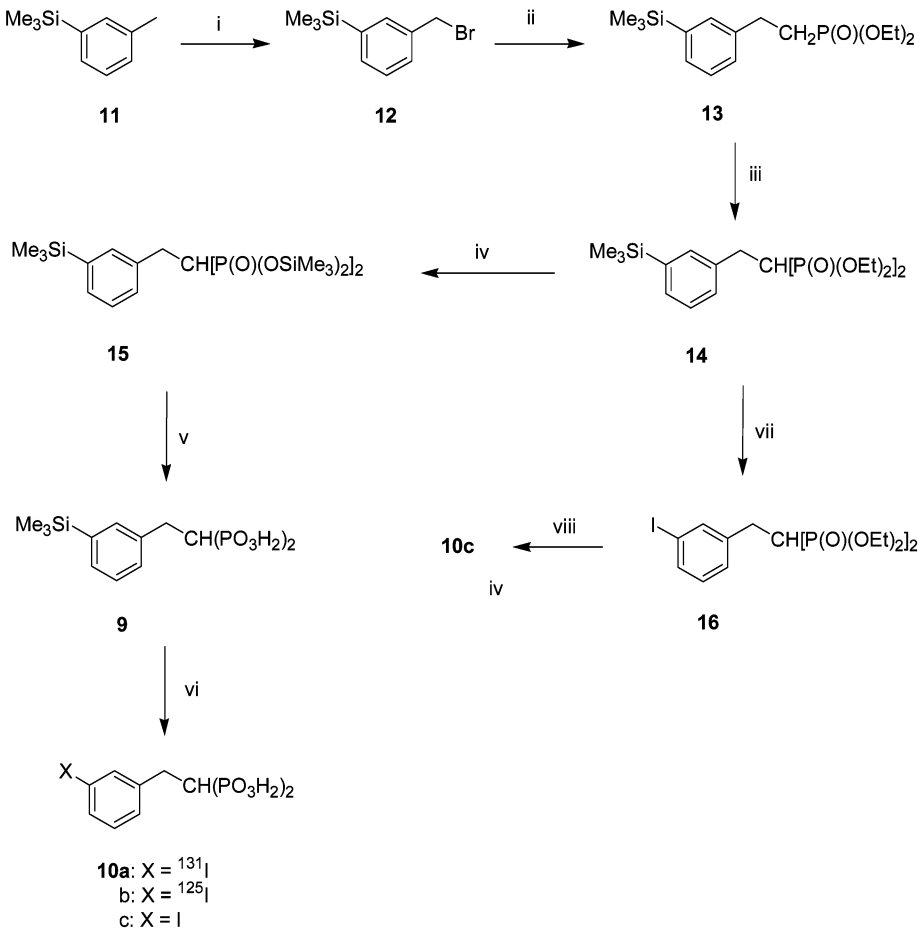
10.1021/jm021107v CCC: $25.00
Published on Web 05/30/2003

Scheme 1[a]



[a] Reagents and conditions: i, NBS, AIBN, PhCl, 80-90 °C; ii, $LiCH_2P(O)(OEt)_2$, THF, −10 °C; iii, LDA, $ClP(O)(OEt)_2$, THF, −78 °C; iv, TMSBr, 20 °C; v, EtOH, H_2O; vi, nca NaI, AcOH, TFA, NCS; vii, ICl, AcOH, 20 °C; viii, (a) TMSBr, 20 °C, (b) EtOH, H_2O.

^{131}I is extensively used for treatment of thyroid cancer.[13] The past decade has also seen an increased use of ^{131}I in radioimmunotherapy (RIT) because of its favorable chemical properties, low cost, and emission of low energy β-particles. In particular, the treatment of non-Hodgkins lymphoma patients has been a success.[14] For similar reasons the incorporation of ^{131}I into a bone-seeking compound, e.g., a bisphosphonate derivative is appealing for the treatment of bone-related cancer.

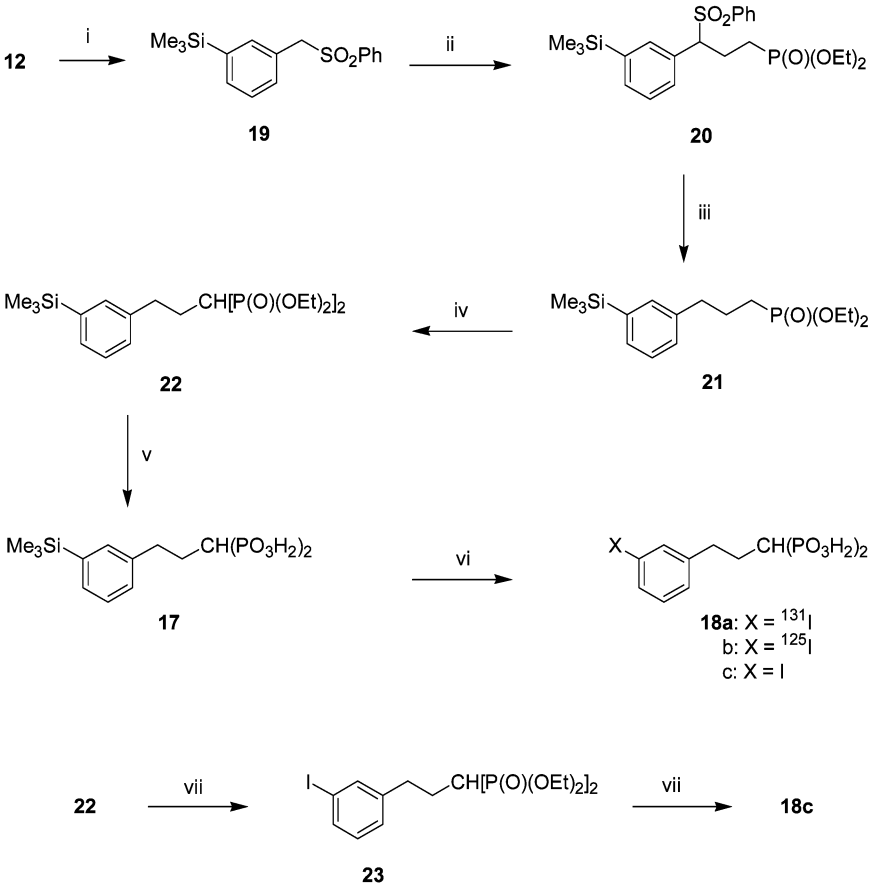
Eisenhut was first to report the potential use of ^{131}I-labeled bisphosphonates for palliative treatment of bone-related cancer.[15,16] In these studies, several benzylidenebisphosphonates (**5**, Chart 1) were synthesized and evaluated. The bone affinity and selectivity of the compounds were found to be comparable to that of other bone-seeking radiopharmaceuticals; however, because of their low in vivo stability these compounds have not been used clinically. Recently, the synthesis of several radiohalogenated bisphosphonates **6** (Chart 1) with improved bone affinity as well as stability was reported.[17,18]

The present study was initiated in order to investigate further the potential use of bisphosphonates for the treatment of bone-related cancer, and herein we report the syntheses and in vivo biological testing of some novel non-carrier-added (nca) [^{125}I]- and [^{131}I]-labeled derivatives of this class of compounds. Furthermore, it was our intention to optimize the molecular structure in order to improve the bone affinity.

Chemistry

It is well established that bisphosphonates accumulate in bone structures. A benzene ring was included in the molecule because we envisioned ways of combining it with radioactive iodine through a halodesilylation reaction. This approach required *m*-trimethylsilylphenylethylidene-1,1-bisphosphonic acid (**9**), and the route to this compound and the iodinated derivatives **10** is outlined in Scheme 1. *m*-Chlorotoluene was transformed to the Grignard reagent in THF, and the reaction with trimethylchlorosilane gave the silane **11** in 92% yield. This was converted to the bromide **12** in 99% yield using NBS and 2,2′-azobis(isobutyronitrile) in chlorobenzene as solvent. Treatment of the bromide with the lithium salt of diethyl methylphosphonate afforded diethyl *m*-trimethylsilylphenylethylphosphonate (**13**) in 53% yield. The phosphonate was then converted to the corresponding lithium salt using 2 equiv of LDA, and the subsequent reaction with diethyl chlorophosphate gave the tetraethyl bisphosphonate **14** in 87% yield. Finally, hydrolysis of this compound was achieved in excellent overall yield by transesterification to the corresponding tetra (trimethylsilyl) ester **15**, followed by addition of aqueous ethanol.[19] The resulting bisphosphonic acid **9** was neutralized with sodium hydroxide and collected as the corresponding disodium salt, which

Scheme 2[a]



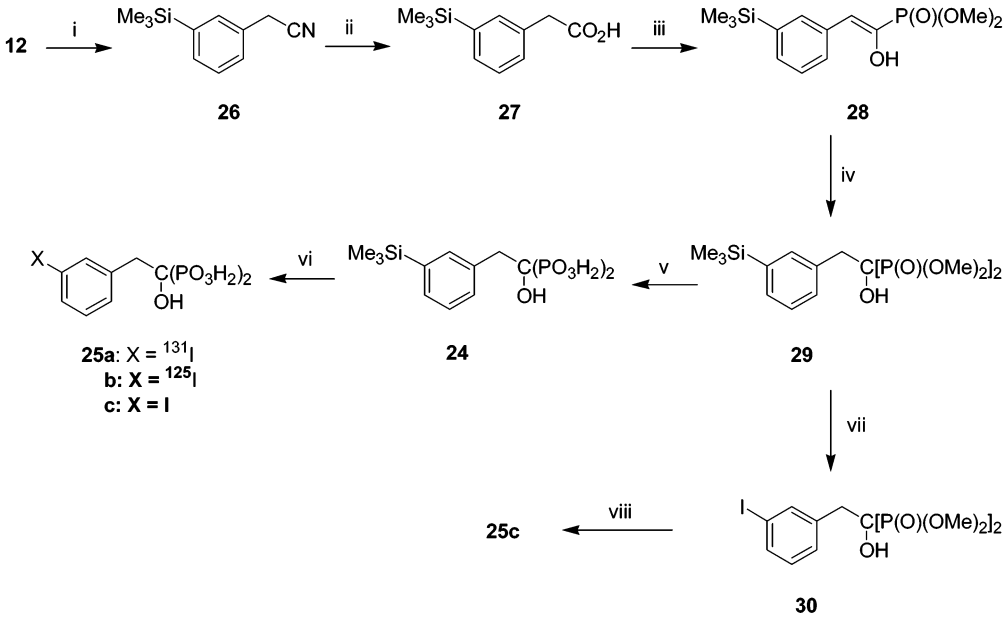
[a] Reagents and conditions: i, (a) NaOH, PhSH, MeOH, (b) Oxone; ii, LDA, $BrCH_2CH_2P(O)(OEt)_2$, THF, −78 °C; iii, Na(Hg), MeOH, 0 °C; iv, LDA, $ClP(O)(OEt)_2$, THF, −78 °C; v, (a) TMSBr, 20 °C, (b) EtOH, H_2O; vi, nca NaI, AcOH, TFA, NCS; vii, ICl, AcOH, 20 °C; viii, HCl, Δ.

was subjected to radiohalogenation using commercially available non-carrier-added [125I]- and the [131I]- sodium iodide. A modification of the oxidative deiodosilylation reaction by Vaidyanathan and Zalutsky[20] was used (NCS, 9:1 TFA:AcOH, 5 min, room temperature) resulting in a radiochemical yield of >95%. Interestingly, labeling could also be carried out in a mixture of acetic acid/urea/water in 70−86% radiochemical yields. The product of the radiolabeling was identified by comparison of the HPLC retention time with that of the corresponding nonradioactive iodide. HPLC was also used to purify the products (see Experimental Section). Thus, using this radiolabeling procedure the [131I]-iodide **10a** and the [125I]-iodide **10b** were obtained. Furthermore, the trimethylsilyl derivative **14** was treated with ICl to give the iodo derivative **16**. Transesterification of this compound resulted in the tetrakis(trimethylsilyl) ester, which upon hydrolysis yielded the iodide **10c**.

The analogue 3-(*m*-trimethylsilylphenyl)propylidene-1,1-bisphosphonic acid (**17**) and its iodinated derivatives **18** were prepared as outlined in Scheme 2. The successive treatment of the bromide **12** with thiophenol and oxone afforded the sulfone **19** in 67% overall yield. Base-induced alkylation of the sulfone with diethyl bromoethylphosphonate furnished the phosphonate **20** in 66% yield. Reductive removal of the benzenesulfonyl group with a large excess of sodium amalgam (10 equiv) in methanol provided the phosphonate **21** in excellent yield, ester hydrolysis being avoided by quenching with saturated aqueous ammonium chloride. The ester was then transesterified and hydrolyzed as described for compound **14**, affording the bisphosphonic acid **17**, which was neutralized and collected as the corresponding disodium salt. The disodium salt of **17** was subjected to radiohalogenation, as described for the analogue **9**, yielding the [131I]-iodide **18a** and the [125I]-iodide **18b**. Reaction of the 1-hydroxy(trimethylsilylphenyl)-bisphosphonate **22** with ICl in acetic acid afforded the iodo ester **23**, which was hydrolyzed to the acid **18c** by refluxing in concentrated hydrochloric acid.

The syntheses of 1-hydroxy(-trimethylsilylphenyl)-ethylidene-1,1-bisphosphonic acid (**24**) and the corresponding iodo derivatives **25** are outlined in Scheme 3. The bromide **12** was converted to *m*-trimethylsilylbenzyl cyanide (**26**) with potassium cyanide under phase transfer conditions.[21] Hydrolysis gave the carboxylic acid **27**, which was converted with thionyl chloride to the acid chloride in good overall yield. Ultrasound assisted reaction of the acid chloride with trimethyl phosphite in THF (0.5 h at 0 °C) proceeded cleanly in close to quantitative yield to give the phosphonate **28**, as a semisolid product. The compound is present as the enol, and the assignment of the *E*-configuration is based on the $^3J_{P-H}$ of 13 Hz in the ^1H NMR spectrum.[22] The subsequent transformation of **28** to the hydroxybisphosphonate **29** was hampered by the phosphonate−phosphate rearrangement, which is both acid- and base-catalyzed.[23] Nicholson and Vaughn reported good yields of tetraalkyl hydroxybisphosphonates from the base-catalyzed condensation of dialkyl phosphites with di-

Scheme 3[a]



[a] Reagents and conditions: i, KCN, Bu_3N; ii, NaOH, MeOH, Δ; iii, $P(OMe)_3$, THF, ultrasound; iv, $HP(O)(OMe)_2$, Bu_2NH; v, (a) TMSBr, 20 °C, (b) EtOH, H_2O; vi, nca NaI, AcOH, TFA, NCS; vii, ICl, AcOH, 20 °C; viii, HCl, Δ.

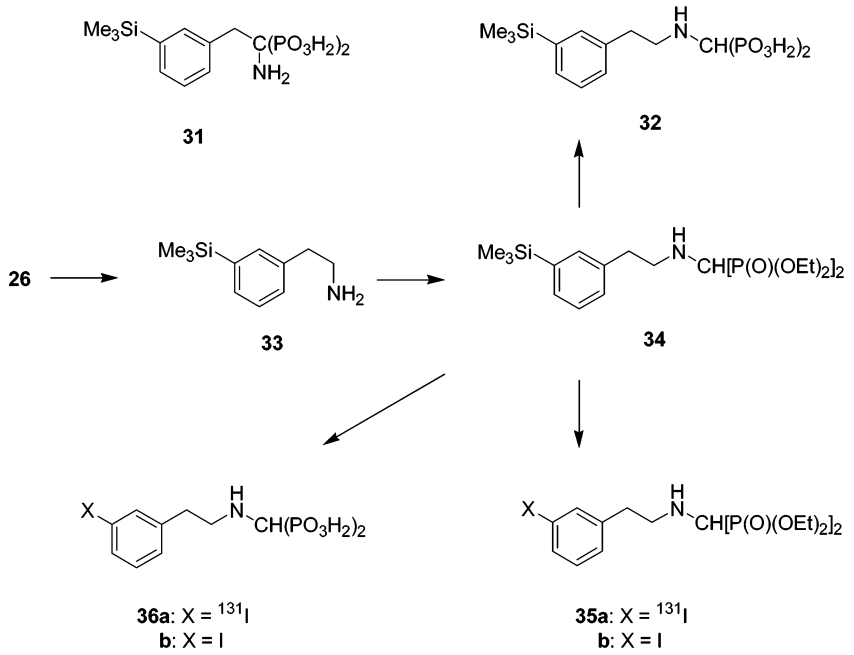
alkyl acylphosphonates when ether was used as solvent;[24] the starting materials are soluble in ether while the hydroxybisphosphonates precipitate during the reaction, thereby reducing the degree of rearrangement. Using ether alone as solvent the hydroxybisphosphonate **29** was obtained in a disappointing 5% yield, but addition of hexane as cosolvent caused precipitation of the bisphosphonate, and the yield of **29** increased to 65%. The latter was transesterified and hydrolyzed as described for compound **14**, affording the bisphosphonic acid **24**, which was neutralized and isolated as the corresponding disodium salt. The disodium salt of **24** was subjected to radiohalogenation, as described for **9**, yielding the [^{131}I]-iodide **25a** and the [^{125}I]-iodide **25b**. Reaction of the bisphosphonate **29** with ICl in acetic acid afforded the iodo ester **30**, which was hydrolyzed to the acid **25c** by refluxing in concentrated hydrochloric acid.

We also wanted to prepare 1-amino-(*m*-trimethylsilylphenyl)ethylidene-1,1-bisphosphonic acid (**31**), as a precursor of the corresponding radioactive iodides. Several routes were tried without success and the difficulties encountered made us change the choice of target compound to *N*-(*m*-trimethylsilylphenylethyl)-aminomethylenebisphosphonic acid (**32**), and its synthesis is depicted in Scheme 4. Treatment of the cyanide **26** with sodium borohydride and zirconium tetrachloride in THF gave the amine **33** in 50% yield,[25] which was converted with triethyl orthoformate and 2 equiv of diethyl phosphite at 150 °C to the aminobisphosphonate ester **34** in 72% yield. Transesterification followed by hydrolysis provided the bisphosphonic acid **32**, which was isolated as the disodium salt. The disodium salt of **32** was subjected to radiohalogenation as described above, yielding the [^{131}I]-iodide **36a.** However, with respect to yields, it was advantageous in this case to convert the ester **34** to the [^{131}I]-iodide **35a**, which was then transesterified and hydrolyzed to the acid **36a**. Furthermore, reaction of the ester **34** with ICl in acetic acid gave the iodide **35b**, which was hydrolyzed to the acid **36b** by refluxing in concentrated hydrochloric acid.

Biology

Biodistribution in Mice. The tissue distribution in mice of **10a, 18a, 25a**, and **36a** is shown in Table 1. Since radioiodine is known to accumulate in the thyroid, high concentration in this organ is indicative of dehalogenation in vivo. Accordingly, tissue samples containing the thyroid were systematically collected and their radioactivity levels measured. Administration of the labeled bisphosphonates resulted in a very low accumulation in the thyroid and in other soft tissue, indicating a high stability in vivo. The bisphosphonate **10a** accumulated fast in bone with high preference. The blood clearance was rapid, and the accumulation in soft tissue was generally low, except for the kidneys. However, the compound was efficiently excreted from this organ, and 24 h after administration the amount of compound present in the kidneys was low as well. There was no significant leakage of the compound from bone tissues, and consequently the resulting femur to soft tissue ratio remained high (>14 after 24 h). The propylidenebisphosphonate **18a** exhibited a similar bone affinity as that of **10a**, and the accumulation in soft tissue was also very low for this compound. A superior bone affinity was recorded for the hydroxybisphosphonate **25a** with an accumulation twice as high as that of **10a**; maximum absorption was reached after 2 h and remained very high after 24 h. The activity in blood was initially high, but had declined 2h after injection. The accumulation in kidney was initially high as well, but clearance from this organ was fairly rapid. A somewhat lower accumulation was found in the liver, and it was negligible in other soft tissue. The tissue distribution of **25a** in rats for a 24 h period is shown in Table 2. The high selectivity and superior bone affinity previously found for this compound, was overall confirmed, with a femur to soft tissue ratio of >45 after 24 h after injection. Surprisingly, the aminomethylenebisphosphonate **36a** appeared to lack bone-seeking properties. Moreover, that compound had a rather high accumula-

Scheme 4[a]



[a] Reagents and conditions: i, $NaBH_4$, $ZrCl_4$, THF; ii, $CH(OEt)_3$, $HP(O)(OEt)_2$, 150 °C; iii, (a) TMSBr 20 °C, (b) EtOH, H_2O; vi, nca NaI, AcOH, TFA, NCS; v, ICl, AcOH, 20 °C; vi, HCl, Δ.

tion in soft tissue. Because of the superior bone affinity of the bisphosphonate **25a**, and its high selectivity, this compound was chosen for further studies.

Pharmacokinetics in Mice. After intravenous injection of **25a** in mice, dynamic studies were performed with a gamma camera equipped with a 6 mm pinhole collimator. The mice were anaesthetized, and their legs and bodies fixed so that the field of view covered the entire animal as seen from the posterior direction. A total of 90 images were obtained during 2h, from which the content in the kidneys, bladder, and the whole body was derived. The analysis showed that, as a mean value, 27% of the injected activity had accumulated in the bladder after 2 h. Furthermore, accumulation in the kidneys reached a maximum 45 min postinjection, amounting to 7.5% of the injected dose at this point of time. From then on the effective halflife in the kidneys was 50 min. The bladder curve was still increasing after 2h. By extrapolating the kidney curves to infinity and assuming that the excreted urine ends up in the bladder, it was estimated that 35% of the initial amount of the radiopharmaceutical left the body. Hence, 65% remained in the body, mainly in the skeleton.

The pharmacokinetics of polyphosphonates in different species, varying from rodents to humans, are known to relate to weight by the expression:[26] corr factor = (mass man/mass animal)$^{0.33}$. In our case, this would mean that the biological processes of uptake and excretion run 15.1 times slower in humans as compared with mice. For the estimation of the cumulative activity in the kidneys, it turns out that the time scale is unimportant, given that the same amount of radioactivity passes through this organ and the excretion curve is exponential.

Dose Estimates. To get an indication of the absorbed radiation dose in different organs in humans, the measured pharmacokinetics of **25a** in mice were extrapolated to humans as follows: It was thus assumed that 65% of the administered radiopharmaceutical is permanently bound to bone surfaces, 10 percent of it in trabecular bone, where it remains until an insignificant amount of ^{131}I is left. This assumption is supported by the data of Table 1, even though the uncertainties are large. Furthermore, it is a general observation that bisphosphonates bind to bone so tightly that the binding can be considered an irreversible process. This observation is corroborated by clinical experience from the use of these compounds in a large number of patients.[27] Additionally, deiodination of the compound when attached to bone is not a very likely process. First, we observed no enhanced enrichment of radioidine in the thyroid region, showing that there is no rapid deiodination of the compound in rats. Second, long-term deiodination is even less likely, since bisphosphonates do not only deposit on bone surfaces, but are also incorporated into the bone matrix as it develops, making the compound out of reach from deiodinase enzymes. An experimental determination of the biological halflife of the compound in humans and possible enrichment of radioiodine in nontarget organs belongs to a future phase I evaluation.

Because of the relatively long half-life of ^{131}I, the uptake phase was neglected in the calculation of residence times. The mouse kidney and bladder curves, extrapolated to infinity and taking into account the differences in time scales between man and mice,[28] were used to estimate the residence times in these organs. Absorbed doses were calculated with the MIRDOSE 3.1 program, assuming an adult patient. To facilitate comparison with dose estimates for other radiopharmaceuticals, values (Table 3) were expressed in both mGy/MBq and rad/mCi.

Survival Studies. The hydroxybisphosphonate **25a** was evaluated in two antitumor efficacy studies in models with human breast cancer cells (MT-1) and

Table 1. Tissue Distributions of nca ^{131}I-Labeled Bisphosphonates in Mice[a]

organ	time postinjection (h)	**10a**	**18a**	**25a**	**36a**
femur	0.5	18.80 ± 0.51		45.03 ± 6.75	
	2	21.16 ± 3.85		45.54 ± 4.10	
	5	10.00 ± 7.81		30.30 ± 8.90	
	24	15.25 ± 4.89	8.84 ± 0.97	37.79 ± 11.78	1.39 ± 0.43
skull	0.5	10.28 ± 2.58		24.61 ± 3.85	
	2	7.64 ± 3.53		26.49 ± 0.61	
	5	6.16 ± 4.82		19.14 ± 8.61	
	24	7.13 ± 2.73	10.36 ± 4.26	21.34 ± 5.62	0.91 ± 0.08
blood	0.5	2.22 ± 0.31		15.92±12.75	
	2	0.31 ± 0.25		1.36 ± 0.25	
	5	0.11 ± 0.09		0.82 ± 0.19	
	24	0.03 ± 0.01	0.02 ± 0.02	0.09 ± 0.03	0.18 ± 0.04
heart	0.5	0.90 ± 0.15		3.35 ± 0.41	
	2	0.26 ± 0.08		0.82 ± 0.21	
	5	0.07 ± 0.05		0.40 ± 0.17	
	24	0.04 ± 0.02	0.06 ± 0.00	0.18 ± 0.07	0.19 ± 0.08
lungs	0.5	1.00 ± 0.12		5.41 ± 1.67	
	2	0.35 ± 0.05		1.20 ± 0.48	
	5	0.10 ± 0.08		0.61 ± 0.05	
	24	0.06 ± 0.03	0.21 ± 0.02	0.54 ± 0.36	0.67 ± 0.19
liver	0.5	2.98 ± 0.65		3.88 ± 0.49	
	2	2.14 ± 0.11		3.36 ± 0.67	
	5	0.94 ± 0.70		2.51 ± 0.07	
	24	0.23 ± 0.02	0.35 ± 0.06	2.27 ± 0.69	5.57 ± 0.79
kidneys	0.5	8.23 ± 6.39		18.64 ± 1.78	
	2	3.68 ± 2.80		13.30 ± 1.70	
	5	2.26 ± 1.64		7.17 ± 0.25	
	24	1.06 ± 0.10	0.85 ± 0.08	5.16 ± 2.00	0.40 ± 0.10
spleen	0.5	0.35 ± 0.26		2.02 ± 0.357	
	2	0.26 ± 0.07		0.77 ± 0.07	
	5	0.12 ± 0.09		0.47 ± 0.06	
	24	0.10 ± 0.03	0.17 ± 0.04	0.49 ± 0.20	2.49 ± 0.37
stomach	0.5	1.14 ± 0.88		3.22 ± 1.06	
	2	1.12 ± 0.50		1.79 ± 0.64	
	5	0.23 ± 0.18		0.38 ± 0.13	
	24	0.05 ± 0.03	0.04 ± 0.01	0.13 ± 0.01	0.07 ± 0.04
intestine	0.5	0.37 ± 0.29		1.07 ± 0.06	
	2	0.28 ± 0.05		1.00 ± 0.20	
	5	0.24 ± 0.17		0.45 ± 0.06	
	24	0.28 ± 0.30	0.05 ± 0.01	0.16 ± 0.05	0.10 ± 0.03
thyroid[b]	0.5	0.21 ± 0.09		0.51 ± 0.19	
	2	0.14 ± 0.05		0.19 ± 0.08	
	5	0.06 ± 0.01		0.49 ± 0.11	
	24	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.04	0.02 ± 0.01

[a] Expressed as % injected dose per gram tissue (mean ± 1 SD for three mice). [b] Expressed as % of injected dose.

Table 2. Tissue Distributions of **25a** in Rats 24 h after Injection[a]

organ	25a
femur	16.44 ± 1.96
skull	5.84 ± 0.35
blood	0.01 ± 0.01
heart	0.04 ± 0.02
lungs	0.05 ± 0.01
liver	0.18 ± 0.03
kidneys	0.36 ± 0.03
spleen	0.09 ± 0.01
stomach	0.05 ± 0.01
intestine	0.03 ± 0.01
thyroid[b]	0.02 ± 0.01

[a] Expressed as % injected dose per gram tissue (mean ± 1 SD for three rats). [b] Expressed as % of injected dose.

Table 3. Estimates of Doses Resulting from Administration of 1.00 GBq of **25a** in Man[a]

target organ	mGy/MBq	rad/mCi
bone surfaces	7.	27.5
red marrow	1.10	4.07
brain	0.249	0.921
kidneys	0.170	0.629
liver	0.106	0.392
urine bladder wall	0.0878	0.325
total body	0.467	1.73

[a] The MIRDOSE (3.1) program was used with the adult phantom, assuming residence times of 28, 153, 10, and 3 h in trabecular bone, cortical bone, kidneys, and the remainder of body, respectively.

human osteosarcoma cells (OHS) in immunodeficient nude rats.

The MT-1 model simulates formation of mixed lytic/sclerotic skeletal metastasis in breast cancer patients.[29] The cell line has an aggressive and metastatic behavior, and animals infected develop tumors in the brain, lung, and adrenals as well. Repeated treatments (days 7 and 14) with the chemotherapeutic agents cisplatin and doxorubicin have not improved survival nor did they have any significant effect on the metastatic growth.[29]

However, while this manuscript was in preparation, a significant antitumor effect was observed after treatment with the α-particle-emitting ^{223}Ra.[30] Treatment with the hydroxybisphosphonate **25a** was initiated 7 days after inoculation of the tumor cells, while the control group received saline only. As shown in Figure 1, the rats in the control group developed tumors and had a mean survival time of 20.8 days (range 20−21, one survivor). Survival time refers to the time from cell inoculation until the animals suffered discomfort because of their metastatic condition and hence were sacrificed. The mean survival times for the animals

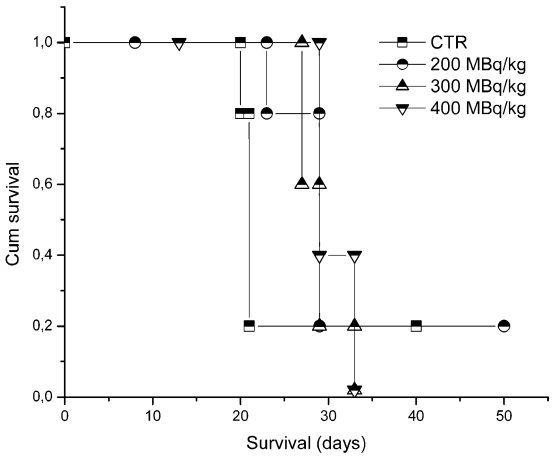


Figure 1. Survival curves for rats injected (LV) with MT-1 cells and treated with bisphosphonate **25a**. A total of 20 animals, allocated in four groups ($n = 5$ in each group), were used. Seven days after cell injection the animals were treated with 200, 300, or 400 MBq/kg of **25a**, whereas the control group received saline only.

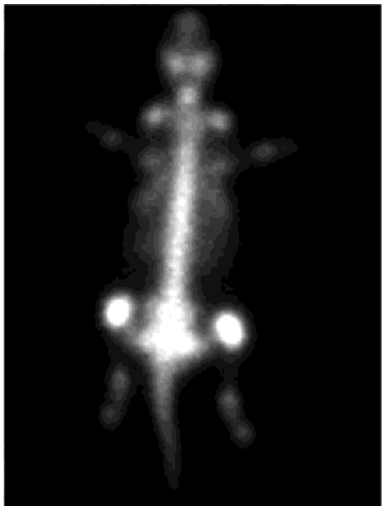


Figure 2. The scintigraphy of a rat (114 g), recorded 24 h after injection of **25a**.

treated with the bisphosphonate **25a** were 27.5, 29.0, and 30.6 days with increasing dose of 200, 300, and 400 MBq/kg, respectively ($n = 5$ in each group). One long time survivor was observed in each of the control groups and the group receiving the lowest dose. This may be interpreted as cases where the cell line failed to establish metastasis in the actual animals, or behaved atypically. Excluding the survivors from the data, the observed survival time for the animals treated with **25a** as compared with the controls are statistically significantly longer ($p < 0.05$, Wilcoxon rank-sum test) at all three dose levels. The results indicate a dose−response relationship as well, but not statistically significant. As part of this experiment, a scintigraphy was obtained from a rat 24 h postinjection. The image shows the favorable targeting abilities of **25a** with particularly high concentration in the spine and the pelvis and no visible presence in soft tissue (Figure 2).

The OHS model was established by intratibial injections of the osteogenic sarcoma (OHS) cell line in immunodeficient rats.[31,32] The model has been used previously to evaluate the antitumor efficacy of several drugs currently used for treatment of bone-related

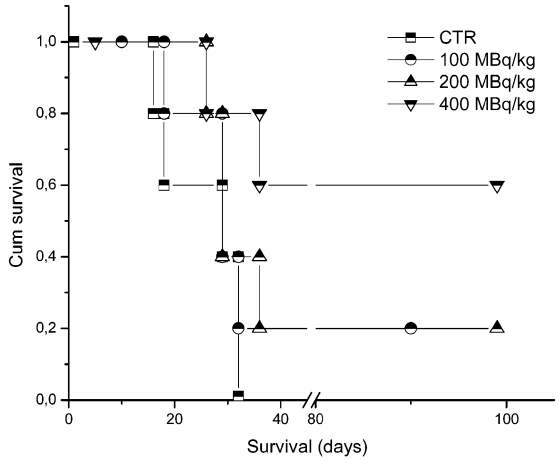


Figure 3. Survival curves for rats inoculated with OHS cells and treated with bisphosphonate **25a**. A total of 20 animals were used, allocated in four groups ($n = 5$ in each group). Seven days after cell inoculation the animals were treated with 100, 200, or 400 MBq/kg of **25a**, whereas the control group received saline only.

cancer. Disease-free latency was defined as the period between tumor cell inoculation and the time the diameter of the tumor-injected tibia had increased by 2−3 mm compared with that of the noninjected contralateral leg. As shown in Figure 3, all the untreated, OHS-injected control animals (5/5) developed palpable bone tumors after 16−32 days (mean 25.4 days). The mean disease-free latency was 27, 30, and 31 days for the animals treated with 100, 200, and 400 MBq/kg of **25a** (day 7), respectively ($n = 5$ in each group). The group receiving the highest dose had a marked increase in disease-free latency time ($P < 0.05$, Kruskal−Wallis test) as compared to the other groups. In addition, 3 of 5 (60%) animals receiving the highest dose were long time survivors. Furthermore, 5 of 15 (33%) animals treated in total with the compound were long time survivors (>100 days), while all controls developed palpable tumors (5/5, <33 days).

Discussion

Only a few ^{131}I-labeled bisphosphonates have so far been prepared, and little is known about the relationship of chemical structure with bone affinity and selectivity. The results of the present study indicate that the nature of the linker between the aromatic group and the bisphosphonate moiety is important; the bone affinity of **10a** is twice that observed for **18a**. Substituting the alpha hydrogen in **10a** with a hydroxyl group as in **25a** doubles the bone affinity as well. When comparing these results with previously reported ^{131}I-labeled bisphosphonates a similar pattern is revealed. To compare bone affinity measured in different species one has to express the uptake as % ID/g tissue × body weight to correct for difference in weight of the animals used. When expressed in this fashion, uptake in femur 24 h after injection was found to be 850 ± 265% in mice, and 985 ± 118% in rats, in the case of **25a**. The corresponding values for [^{89}Sr] strontium chloride and [^{153}Sm] samarium EDTMP are 360% (24 h, mice)[30] and 580% (24 h, rats),[33] respectively. The analogue of **25a** lacking the methylene linker has been reported to have an uptake in femur (47 h, rats) of 300%.[34] Recently, Larsen

et al.[18] reported the biodistribution data for a related ^{131}I-labeled hydroxy bisphosphonates, i.e., compound **6a**. In this study uptake in femur (24 h, mice) was reported to be 415%, substantiating the importance of the linker in relation to bone affinity. The same authors also found a reduction in bone affinity when the phenyl group in **6a** was replaced by a pyridine ring as in compound **6b**.[18] Why the amino bisphosphonate **36a** lack bone-seeking abilities is not understood.

The dosimetry part of this work only serves to indicate what might be expected in humans. Per unit of injected activity, the bone marrow dose is proportional to the fraction of injected activity that is accumulated in the skeleton, and so is the dose to a tumor. An important unknown factor is the ratio between uptake of the compound in the new bone matrix surrounding a tumor and the normal bone, including trabecular bones adjacent to the bone marrow. A rapid and high bone uptake means that a lower fraction of the activity passes through the kidneys and the bladder and thus results in lower doses to these organs. The kidney data of Table 1 indicate that the kidney excretion may be slower than what was measured in vivo. This would give a higher dose to the kidneys than indicated by Table 3, perhaps by as much as a factor 2. Nevertheless, this would still amount to only 4% of the dose to the bone surfaces. Proper dosimetric studies should be based on measurements in humans with ^{123}I and are beyond the scope of this article.

The results from the survival study involving the OHS cell line indicate that the antitumor efficacy of 400 MBq/kg of the hydroxybisphosphonate **25a** is similar to that of 800 MBq/kg of [^{153}Sm] samarium−EDTMP.[35] However, the actual dose to bone marrow is estimated to be 2.2−3.6 times as high for 800 MBq/kg [^{153}Sm] samarium−EDTMP as compared to 400 MBq/kg of the hydroxybisphosphonate **25a**. Consequently, it is reasonable to assume that considerably higher doses of **25a** may be delivered to tumor volumes without increased detrimental effects as compared with currently used drugs.

One must recognize that the optimal choice of radionuclide depends on the geometrical relation between the structures that accumulate the radiopharmaceutical and the tumor itself (e.g., uptake in a bone reaction zone surrounding a metastatic lesion). Uptake in the tumor will only happen in the case of osteosarcoma. Tumor penetration and cell killing ability of the radiation depend on the types of radiation emitted. Thus, a large metastatic lesion would require even higher energy beta particles than those emitted by ^{131}I. If the radionuclide is uniformly distributed within an osteosarcoma, a low beta energy would lead to deposition of most of the released energy within the tumor. Alpha emitters are only useful it they are accumulated in the tumor or in a zone immediately adjacent to a very small tumor. Furthermore, it should be noted that the bone marrow compartments and the bones in general are significantly smaller in rats than in humans. Hence it is possible that the results of the survival studies reflect dose contributions to the tumorous sites from activity deposited in cortical bone. Because of the larger dimensions, this would seldom be the case in humans, where the effect of small tumors probably would mainly relate to the uptake in a reaction zone in the bone marrow surrounding the tumor.

To the best of our knowledge, compound **25a** has the highest bone-affinity reported for any radiopharmaceutical. The radionuclide employed is readily available at reasonable cost, and the method developed for radiohalogenation provides close to quantitative radiochemical yields in short time with a mild and safe handling procedure. Most importantly, dose estimates as well as preliminary survival studies indicate the improved antitumor efficacy of **25a** compared with currently used bone-seeking radiopharmaceuticals.

Experimental Section

Chemistry. General. Unless otherwise indicated analytical grade reagents were used without further purification. Other grades were purified according to standard procedures before use. All experiments involving organic solvents were run under an argon atmosphere. NMR spectra were obtained with Varian Gemini-200, Bruker DPX 200, and Bruker DRX 500 instruments. Infrared spectra were obtained with a Perkin-Elmer 1310 infrared spectrophotometer or a Nicolet Magna-IR 550 spectrometer. Mass spectra were obtained on a Fision VG pro spectrometer; for GC-MS, a Fisons 8065 gas chromatograph with a CP SIL SCB-MS column was attached to the spectrometer. Radioactivity measurements were carried out either with a Beckman LS 6500 liquid scintillation counter or with a Capintec CRC-7R radioisotope calibrator. Sodium [^{131}I]-iodide was obtained from NEN Life Science Products, and sodium [^{125}I]-iodide was purchased from Amersham, UK. HPLC separations were performed with a system consisting of a LC10 AT pump and an SPD-M10A diode array UV detector (both Shimadzu) combined with a Beckman Model 170 radioactivity detector. The columns used were PLRP−S (5 μm, 100 Å, 150 × 1.6 mm and 8 μm, 1000 Å, 150 × 4.6 mm, Polymer Laboratories, UK). Sonification experiments were carried out with a Transsonic 310 ultrasound bath (Heigar). Melting points were measured on a Büchi apparatus and are uncorrected.

General Procedures. Iodination. The trimethylsilyl derivative (*x* mmol) was added to a solution of ICl (2*x* mmol) in AcOH (5*x* mL), and the mixture was stirred for 5 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in ether. The solution was washed successively with saturated $NaHCO_3$ (aq), saturated Na_2SO_3 (aq), water, and brine. The organic phase was dried ($MgSO_4$) and the ether removed under reduced pressure to give the iodo derivative. **Transesterification**. To 1 equiv of the parent tetraalkyl bisphosphonate was added 10 equiv of TMSBr, and the mixture was stirred for 3 h at room temperature. The volatiles were then removed under reduced pressure to give the tetrakis(trimethylsilyl) ester. **Hydrolysis**. The tetraalkyl bisphosphonate was added to concentrated HCl and the mixture heated under reflux for 3 h. The reaction mixture was then concentrated to dryness under reduced pressure to give the bisphosphonic acid.

***m*-Trimethylsilyltoluene (11).** A solution of *m*-chlorotoluene (12.66 g, 0.10 mol) and 1,2-dibromoethane (0.5 mL) in THF (20 mL) was added to magnesium turnings (2.67 g, 0.11 mol). The mixture was heated under reflux for 4 h, after which the heating bath was removed and trimethylchlorosilane (11.08 g, 0.10 mol) was added at a rate maintaining gentle reflux. The mixture was stirred for another 0.5 h, and 5% aq $NaHCO_3$ (100 mL) was added. The resulting mixture was extracted with CH_2Cl_2, and the extract was washed with brine and dried ($MgSO_4$). Filtration and evaporation provided 15.05 g (92%) of the silane **11** as a pale, yellow-colored oil. Spectroscopic data were identical to those of an authentic sample.[36]

***m*-Trimethylsilylbenzyl Bromide (12).** A mixture of the silane **11** (8.22 g, 50 mmol), NBS (8.90 g, 50 mmol), AIBN (0.05 g), and chlorobenzene (50 mL) was heated in an oil bath kept at 110 °C. When the reaction mixture reached 80−90 °C, an

exothermic reaction started and the orange suspension became a colorless solution within minutes. The mixture was cooled with the aid of an ice-bath, ice−water was added, and the resulting mixture was extracted with hexane. The organic phase was washed with water and brine and dried ($MgSO_4$). Filtration through a silica plug (10 g) followed by evaporation of solvents yielded 12.05 g (99%) of the bromide **12** as an oil. Spectroscopic data were identical with those of an authentic sample.[37]

Diethyl *m*-Trimethylsilylphenylethylphosphonate (13). To a solution of diethyl methylphosphonate (2.28 g, 15 mmol) in THF (20 mL), kept at −78 °C, was added BuLi (17 mmol, 1.6 M solution in hexane). After 45 min, the white suspension was added to a cold (−78 °C) solution of the bromide **12** (3.65 g, 15 mmol) in THF (20 mL), and the mixture was stirred for 1.5 h. The temperature was then increased to −10 °C, and after 1 h the deeply red-colored solution was quenched with AcOH (1 mL). Volatiles were removed under reduced pressure, and the residue was purified by flash chromatography (silica, EtOAc) to give 2.48 g (53%) of the phosphonate **13** as a colorless oil. IR (film): 3473, 2955, 1248, 1058, 1031 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.30 (9H, s), 1.31 (6H, t, $J = 7$ Hz), 2.10 (2H, m), 2.92 (2H, m), 4.11 (4H, m), 7.29 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 1.2, 16.4, 26.3, 28.6, 29.0, 61.5, 127.9, 128.4, 131.2, 132.9, 139.9, 140.2, 140.7; HRMS-EI (M^+) *m/z* 314.1453 (calculated for $C_{15}H_{27}O_3PSi$ 314.1467).

Tetraethyl *m*-Trimethylsilylphenylethylidene-1,1-bisphosphonate (14). A solution of the phosphonate **13** (2.51 g, 8 mmol) in THF (10 mL) was added to a solution of LDA (16 mmol) at −78 C. After 30 min, diethyl chlorophosphate (1.41 g, 9 mmol) was added, and the mixture was stirred for 45 min. The temperature was then increased to −25 °C, and the reaction mixture was quenched by addition of saturated NH_4Cl (aq). The mixture was concentrated under reduced pressure, and the residue was extracted with hexane. The extract was washed with water and brine and dried ($MgSO_4$). Filtration and evaporation provided 3.22 g (87%) of the bisphosphonate **14** as a pale yellow-colored oil. ^1H NMR (200 MHz, $CDCl_3$): 0.19 (9 H, s), 1.21 (12 H, m), 2.61 (1 H, m), 3.19 (2 H, m), 4.03 (8 H, m), 7.26 (4 H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 0.5, 17.6, 32.5, 37.7, 40.3, 42.9, 63.4, 127.9, 129.6, 131.7, 134.0, 138.8, 138.9, 139.1, 140.4; HRMS-EI (M^+) *m/z* 450.1753 (calculated for $C_{19}H_{36}O_6P_2Si$ 450.1756).

***m*-Trimethylsilylphenylethylidene-1,1-bisphosphonic Acid (9).** Transesterification of the ester **14** (0.45 g, 1.0 mmol) was carried out as described under general procedures. The resulting tetrakis(trimethylsilyl) ester was dissolved in EtOH (90%, 2.0 mL) at 0 °C and stirred for 30 min. NaOH (0.08 g, 2 mmol) in EtOH (90%, 4 mL) was added, and removal of volatiles provided **9** as a white solid (0.43 g).

Tetraethyl *m*-Iodophenylethylidene-1,1-bisphosphonate (16). Iodination of the ester **14** was carried out as described under general procedures. IR (film): 3350, 2960, 1240, 1015, 960 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 1.26 (12H, m), 2.57 (1H, m), 3.12 (2H, m), 4.07 (8H, m), 7.39 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 16.3, 30.7, 36.2, 38.9, 41.5, 62.5, 93.9, 128.1, 129.8, 135.4, 137.8, 141.9; HRMS-EI (M^+): *m/z* 504.0365 (calculated for $C_{16}H_{27}O_6P_2I$ 504.0328).

***m*-Iodophenylethylidene-1,1-bisphosphonic Acid (10c).** The ester **16** was transesterified as described under general procedures. Hydrolysis was achieved by stirring the resulting tetrakis(trimethylsilyl) ester in EtOH (75%) overnight.

***m*-Trimethylsilylbenzyl phenyl sulfone (19).** To a solution of NaOH (0.80 g, 20 mmol) in MeOH (20 mL) was added thiophenol (2.20 g, 20 mmol). After 10 min was added a solution of the bromide **12** (4.38 g, 18 mmol) in MeOH (10 mL), and the resulting mixture was stirred overnight. The crude product was separated between water and EtOAc, and the extract was washed with water and brine and dried ($MgSO_4$). Filtration and evaporation yielded the sulfide (4.79 g, 98%) as a pale, yellow-colored oil. The product (2.73 g, 10 mmol) was dissolved in MeOH (40 mL), and a suspension of oxone (18.44 g, 30 mmol) in water (40 mL) was added at 0 °C. The reaction mixture was stirred at ambient temperature overnight. The resulting mixture was extracted with CH_2Cl_2, and the extract washed with brine and dried ($MgSO_4$). Filtration and evaporation gave a solid residue (2.84 g), which was purified by chromatography on silica (hexane/EtOAc gradient), furnishing the sulfone **19** (2.05 g, 67%) as a white solid, mp 73−74 °C. IR (neat): 2954, 1446, 1310, 1253, 1155 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.18 (9H, s), 4.35 (2H, s), 7.31 (9, m); ^{13}C NMR (50 MHz, $CDCl_3$): −1.2, 63.1, 127.6, 128.1, 128.8, 128.9, 131.4, 133.7, 135.7, 137.9, 141.1; HRMS-EI (M^+): *m/z* 304.0943 (calculated for $C_{16}H_{20}O_2SiS$ 304.0953).

Diethyl 3-(*m*-Trimethylsilylphenyl)-3-benzenesulfonylpropyl-1-phosphonate (20). To a solution of the sulfone **19** (3.04 g, 10 mmol) in THF (50 mL) was added LDA (10.5 mmol) at −78 °C. The mixture was stirred for 0.5 h, and diethyl 2-bromoethylphosphonate (2.45 g, 10 mmol) was then added neat. After 2 h, the reaction was quenched with AcOH (1 mL) at 0 °C. Concentration under reduced pressure followed by chromatography (silica, EtOAc) of the residue gave the phosphonate **20** (3.08 g, 66%) as a colorless oil. IR (film): 2982, 2956, 1308, 1249, 1056 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.13 (9H, s), 1.27 (6H, m), 1.70 (2, m), 4.09 (5H, m) and 7.20 (9H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 1.4, 16.3 (d, $J = 5$ Hz), 20.8, 21.5, 24.4, 61.7 (d, $J = 7$ Hz), 70.9, 71.2, 128.0, 128.5, 128.9, 129.5, 130.5, 133.4, 133.8, 135.1, 137.0, 140.9; HRMS-ESI (M + 1^+): *m/z* 469.1628 (calculated for $C_{22}H_{34}O_5SiP$ 469.1621); HPLC: (PLRP 100Å, 95:5 MeOH/H_2O), tR =3.5 min, >95% purity.

Diethyl 3-(*m*-Trimethylsilylphenyl)propyl-1-phosphonate (21). To a solution of the phosphonate **20** (1.15 g, 2.45 mmol) in MeOH (25 mL) at 0 °C was added Na_2HPO_4 (3.48 g, 24.5 mmol) followed by Na(Hg) 6% (9.21 g, 24.5 mmol). The resulting mixture was stirred for 3 h and quenched with saturated NH_4Cl (aq). The mixture was extracted with ether, and the extract was washed with brine and dried ($MgSO_4$). Evaporation under reduced pressure provided the phosphonate **21** (0.77 g, 96%) as an oil. IR (film): 2955, 1405, 1259, 1060, 1032 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.28 (9H, s), 1.33 (6H, t, $J = 7$ Hz), 1.86 (4H, m), 2.73 (2H, t, $J = 7$ Hz), 4.10 (4H, m), 7.28 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 1.0, 16.5, 24.3, 25.2 (d, $J = 141$ Hz), 36.7 (d, $J = 17$ Hz), 61.5, 127.9, 129.0, 131.2, 133.5, 140.3, 140.7; HRMS-EI (M^+): *m/z* 328.1634 (calculated for $C_{16}H_{29}O_3P$ 328.1624); HPLC: (PLRP 100 Å, 95:5 MeOH/H_2O), $t_R = 4.2$ min, >95% purity

Tetraethyl 3-(*m*-Trimethylsilylphenyl)propyl-1,1-bisphosphonate (22). To LDA (8.9 mmol) was added a solution of the phosphonate **21** (1.40 g, 4.25 mmol) in THF (15 mL) at −78 °C. After 0.5 h, diethyl chlorophosphate (0.70 g, 4.5 mmol) was added, and the mixture stirred for 2 h. The reaction was quenched with AcOH (0.5 mL), and the resulting mixture was evaporated to dryness under reduced pressure. Flash chromatography (silica, Et_2O/EtOH gradient) of the residue gave the bisphosphonate **22 (**1.86 g, 94%) as colorless oil. IR (film): 2981, 1249, 1027, 969 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.25 (9H, s), 1.32 (12H, m), 2.29 (3H, m), 2.91 (2H, t, $J = 7$ Hz), 4.17 (8H, m) and 7.29 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 1.3, 16.2, 27.2, 34.6, 35.6 (t, $J = 134$ Hz), 62.4, 127.7, 128.9, 131.0, 133.4, 140.0, 140.4; HRMS-EI (M^+): *m/z* 464.1878 (calculated for $C_{20}H_{38}O_6P_2Si$ 464.1913).

Tetraethyl 3-(*m*-Iodophenyl)propyl-1,1-bisphosphonate (23). The bisphosphonate **22** (0.46 g, 1 mmol) was iodinated as described under general procedures to provide the bisphosphonate **23** (0.46 g, 86%) as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$): 1.26 (12H, m), 2.14 (3H, m), 2.28 (2H, t, $J = 7$ Hz), 4.08 (8H, m), 7.20 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 6.3, 26.9, 33.9, 35.5 (t, $J = 133$ Hz), 62.4, 94.3, 127.8, 130.0, 135.1, 137.5, 143.2; HRMS-EI (M^+): *m/z* 518.0460 (calculated for $C_{17}H_{29}O_6P_2I$ 518.0484).

3-(*m*-Trimethylsilylphenyl)propylidene-1,1-bisphosphonic Acid (17). The ester **22** (0.23 g, 0.5 mmol) was transesterified as described under general procedures. The tetrakis(trimethylsilyl) ester was then dissolved in EtOH (75%, 1.5 mL) at 0 °C. After 0.5 h, a solution of NaOH (40 mg, 1 mmol) in EtOH (75%, 1 mL) was added. Evaporation under

reduced pressure furnished the disodium salt of **17** (0.20 g) as a white solid.

3-(*m*-Iodophenyl)propylidene-1,1-bisphosphonic Acid (18c). The ester **23** was hydrolyzed as described under general procedures to give the acid **18c** as a tanned solid.

***m*-Trimethylsilylbenzyl Cyanide (26).** A mixture of KCN (5.5 g, 84 mmol), the bromide **12** (5.31 g, 21.8 mmol), tributylamine (0.11 g, 0.59 mmol), and water (12.5 mL) was stirred overnight. The product was extracted with CH_2Cl_2, the extract was filtered through a silica plug (5 g), and solvents were removed under reduced pressure. The residue was distilled to give the cyanide **26** (2.27 g, 55%), bp 134−136 °C (2 mm). IR (film): 2956, 2255, 1412, 1249, 885, 867 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.31 (9H, s), 3.76 (2H, s), 7.42 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 0.3, 24.3, 117.5, 127.7, 127.9, 128.6, 132.0, 132.3, 141.2; HRMS-EI (M^+): *m*/*z* 189.0972 (calculated for $C_{11}H_{15}NSi$ 189.0974).

***m*-Trimethylsilylphenylacetic Acid (27).** To a solution of NaOH (4.0 g, 0.1 mol) in MeOH (20 mL) was added the cyanide **26** (2.84 g, 15.0 mmol), and the mixture was heated under reflux for 4 h. The solvent was evaporated, and the residue was dissolved in water (50 mL). The resulting solution was washed with CH_2Cl_2, acidified with 85% H_3PO_4 to pH 2, and extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). Solvents were removed under reduced pressure to give the acid **27** (2.93 g, 94%) as a yellow-colored oil. IR (film): 3600−2600, 1717, 1418, 1254, 847 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.32 (9H, s), 3.70 (2H, s), 7.42 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 0.5, 41.6, 127.8, 129.6, 132.1, 132.3, 134.1, 140.8, 177.7; HRMS-EI (M^+): *m*/*z* 208.0918 (calculated for $C_{11}H_{16}O_2Si$ 208.0920).

Dimethyl *m*-Trimethylsilylphenylacetylphosphonate (28). A mixture of the acid **27** (1.04 g, 5.0 mmol) and $SOCl_2$ (0.82 g, 6.9 mmol) was stirred at room-temperature overnight. Toluene (2 mL) was added, and volatiles were removed under reduced pressure. The residue was dissolved in THF (5 mL), and trimethyl phosphite (0.69 g, 5.5 mmol) was added at −20 °C. The resulting solution was kept in an ultrasound bath at 0 °C for 30 min. Evaporation of solvent gave the crude bisphosphonate **28** (1.63 g, 99%), which consisted essentially of the *E*-enolate. The unstable product was used further without purification. An analytical sample was prepared by washing the crude product with hexane. IR (film): 3600−2600, 1698, 1249, 1036 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.19 (9H, s), 3.86 (6H, d, J = 14 Hz), 6.11 (1H, d, J = 13 Hz), 7.46 (4H, m), 7.92 (1H, d, J = 7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): 0.7, 54.6, 117.5, 118.1, 128.1, 130.4, 132.9, 133.6, 134.0, 134.9, 138.3, 140.6, 142.3; P (202 MHz; $CDCl_3$; H_3PO_4): 16.3; HRMS-EI (M^+): *m*/*z* 300.0929 (calculated for $C_{13}H_{21}O_4PSi$ 300.0947).

Tetramethyl 1-Hydroxy(*m*-trimethylsilylphenyl)ethylidene-1,1-bisphosphonate (29). To an ice-cold solution of the phosphonate **28** (1.63 g, 5.0 mmol) in ether (10 mL) was added a solution of dimethyl phosphite (0.83 g, 7.5 mmol) and dibutylamine (0.32 g, 2.5 mmol) in hexane (10 mL). A white solid formed after a few minutes. After 20 min, the mixture was filtered to give the bisphosphonate **29** (1.34 g, 65%) as a white solid, mp 114−118 °C. IR (film): 3700−2815, 2958, 1645, 1251 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.25 (9H, s), 3.38 (2H, t, J = 14 Hz), 3.76 (12H, m), 7.39 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 0.1, 40.1, 54.9, 73.3, 76.3, 79.3, 127.7, 132.1, 132.4, 133.8, 136.6, 140.2; P (202 MHz; $CDCl_3$; H_3PO_4): 20.7; HRMS-EI (M^+): *m*/*z* 410.1080 (calculated for $C_{15}H_{28}O_7P_2Si$ 410.1080).

1-Hydroxy(*m*-trimethylsilylphenyl)ethylidene-1,1-bisphosphonic Acid (24). The ester **29** (0.82 g, 2.0 mmol) was transesterified as described under general procedures. The resultant tetrakis(trimethylsilyl) ester was dissolved in EtOH (75%, 8 mL) at 0 °C and stirred for 0.5 h. The mixture was neutralized by addition of Na_2CO_3 (aq) (1.0 M, 3 mL) and concentrated under reduced pressure to give the disodium salt of **24** (0.75 g), as a white solid.

Tetramethyl 1-Hydroxy(*m*-iodophenyl)ethylidene-1,1-bisphosphonate (30). The bisphosphonate **29** (0.41 g, 1.0 mmol) was added to a solution of ICl (0.32 g, 2.0 mmol) in acetic acid (2 mL), and the solution was stirred for 5 h. The solvent was evaporated, and the residue was extracted with 1:1 hexane/ether (2 × 10 mL). Concentration of the extract under reduced pressure furnished the bisphosphonate **30** (0.38 g, 81%) as an orange-colored oil. IR (film): 3381 (br), 2958, 1643, 1216, 1057 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 3.18 (2H, t, J = 14 Hz) 3.74 (12H, m), 7.32 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 39.7, 55.8, 56.3, 73.3, 76.3, 79.3, 94.2, 129.9, 130.8, 136.3, 136.8, 140.2; P (500 MHz; $CDCl_3$; H_3PO_4): 19.6; HRMS-EI(M^+): *m*/*z* 463.9630 (calculated for $C_{12}H_{19}O_7P_2I$ 463.9651).

1-Hydroxy(*m*-iodophenyl)ethylidene-1,1-bisphosphonic Acid (25c). The bisphosphonate **30** was hydrolyzed as described under general procedures to give the title compound as an orange colored solid.

***m*-Trimethylsilylphenylethylamine (33)**. To a suspension of $ZrCl_4$ (4.66 g, 20 mmol) in THF (70 mL) was added $NaBH_4$ (3.03 g, 80 mmol). This resulted in gas evolution and the formation of a cream-colored suspension. The cyanide **26** (3.03 g, 16 mmol) was added, and the mixture was stirred for 24 h. After cooling in ice, the reaction was quenched by addition of water, then 25% NH_3 (aq) until basic and extracted with EtOAc. The organic phase was washed with brine, and the solvents were removed under reduced pressure to give crude amine (3.65 g), which was purified by precipitation of the picric salt from benzene. The salt was dissolved in water, LiOH was added until pH > 11, and the aqueous phase was extracted with ether. The picric acid was removed by repeatedly washings with NaOH (aq). The organic phase was washed with brine and dried ($MgSO_4$), and the solvents were evaporated to give the amine **33** (1.53 g, 50%) as pale, yellow-colored oil. IR (film): 2954, 1248, 858 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 0.27 (9H, s), 1.49 (2H, br, NH2), 2.76 (2H, d, J = 7 Hz), 2.98 (2H, d, J = 7 Hz), 7.28 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): 0.37, 40.4, 43.8, 127.0, 128.4, 130.3, 133.8, 138.0, 139.6; HRMS-EI(M^+): *m*/*z* 193.1293 (calculated for $C_{11}H_{19}NSi$ 193.1287).

***N*-(*m*-Trimethylsilylphenylethyl)aminomethylenebisphosphonic Acid (32)**. The bisphosphonate **34** (0.48 g, 1 mmol) was transesterified by the general procedure. The resultant tetrakis(trimethylsilyl) ester was dissolved in a mixture of CH_3CN (2 mL) and MeOH (0.5 mL), and the solution was left in the cold for 5 h. A solution of NaOH (0.08 g, 2 mmol) in EtOH (2 mL) was then added. The resulting precipitate was collected by filtration and dried under reduced pressure to give the disodium salt of **32** (0.38 g) as a white solid.

Tetraethyl *N*-(*m*-Iodophenylethyl)aminomethylenebisphosphonate (35). The trimethylsilyl derivative **34** was iodinated with ICl by the general procedure to give the iodo ester **35**. IR (film): 2982, 1658, 1247, 1042 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 1.29 (12H, m), 2.67 (2H, t, J = 7 Hz), 3.06 (2H, m), 3.23 (1H, t, J = 22 Hz), 4.14 (8H, m), 7.24 (4H, m, Ar); ^{13}C NMR (50 MHz, $CDCl_3$): 16.1, 35.8, 50.8, 51.0, 53.9, 56.8, 63.0, 94.0, 127.7, 129.7, 134.8, 137.2, 141.7; HRMS-ESI ($M + 1^+$): *m*/*z* 534.0666 (calculated for $C_{17}H_{31}NO_6P_2I$ 534.0668).

***N*-(*m*-Iodophenylethyl)aminomethylenebisphosphonic Acid (36b).** Hydrolysis of the iodo ester **35** with concentrated HCl, as described under general procedures, furnished the acid **36b**.

Preparation of [^{125}I and ^{131}I] 10a, 10b, 18a, 18b, 25a, and 25b. To a solution of the radionuclide in water (2 μL) was added a solution of NCS (400 μg, 3 μmol) in TFA (10 μL), followed by a solution of the appropriate trimethylsilyl bisphosphonate (0.2 μmol) in AcOH (2 μL). The mixture was sealed, swirled, and left for 5 min at room temperature. In the case of the hydroxy compound **25a** a trace of TFA was required to dissolve the compound in AcOH.

Purification of [^{125}I and ^{131}I] 10a and 10b. The reaction mixture was purified by means of HPLC. For this purpose a PLRP−S column (100Å) was used with a mobile phase consisting of 50 mM phosphate buffer with pH 7.2, to which 2% EtOH was added. With a flow rate of 1 mL/min the iodides **10a** and **10b** eluted after 6.0 min. The radiochemical yield, as determined by integration, was >95%.

Purification of [^{131}I and ^{125}I] 18a and 18b. The reaction mixture was purified by means of HPLC. A PLRP−S column (1000Å) was employed with a mobile phase consisting of 50 mM phosphate buffer with pH 7.3, to which 2% EtOH was added. With a flow rate of 1 mL/min the iodides **18a** and **18b** eluted after 5.9 min. The radiochemical yield as determined by integration, was >95%.

Purification of [^{131}I and ^{125}I] 25a and 25b. Phosphoric acid (25%, 30μL) was added to the solution of the crude labeled compound, and the mixture was placed in an ultrasound bath for 5 min. HPLC was carried out with a PLRP−S column (5 μm, 100Å) and a mobile phase consisting of 50 mM phosphate buffer with pH of 7.1, to which 2% EtOH was added. With a flow rate of 1 mL/min, the iodides **25a** and **25b** eluted after 4.9 min. The radiochemical yield as determined by integration, was >95%.

Preparation and Purification of [^{131}I] 36a. To a solution of Na^{131}I in water (2 μL) was added a solution of NCS (400 μg) in TFA (10 μL), followed by addition of the ester **34** (100 μg, 0.2 μmol) in AcOH (2 μL). The resulting mixture was sealed, swirled, and left for 0.5 h. Analysis and purification was carried out by means of HPLC. For this purpose a PLRP−S (1000Å) column was used, and the mobile phase consisted of 70:30:1 MeOH/H_2O/TFA. With a flow rate of 1 mL/min, the iodide **35a** eluted after 6.8 min. The yield, as measured by integration, was 50%. The iodide was collected and the eluate concentrated to dryness. The residue was dissolved in TMSBr (2.5 mL) and left overnight. Evaporation to dryness, followed by addition of 75% EtOH (0.25 mL), provided the iodo acid **36a**. With a flow rate of 1 mL/min the iodide **36a** eluted after 2.7 min.

Biology. Animals. Biodistribution and antitumor efficacy studies were performed in healthy athymic (nude) mice (balb/c, nu/nu) and congenitally athymic (nude) rats (Han *rnu*/*rnu* Rowett) of both sexes. The weight of the animals is specified for each experiment. The animals were anaesthetized with of a mixture with equal parts of fentanyl/fluanison (Hypnorm, Janssen, Beerse, Belgium) and midazolam (Dormicum, Roche, Basel, Switzerland) (0.3 mL/100 g for rats and 0.1 mL/10 g for mice). The animals were bred in the nude rodent facility at the Norwegian Radium Hospital, maintained under specific pathogen-free conditions, and food and water were supplied ad libitum. Housing and all procedures involving animals were performed according to protocols approved by the animal care and use committee, in compliance with the National Committee for Animal Experiment's guidelines on animal welfare.

Biodistribution. The radiolabeled compounds were evaluated in healthy mice (20−25 g) and rats (60 g). For each mouse, 50−400 KBq in 100 μL of phosphate buffer, pH 6−7, was injected via a tail vein, and three animals were sacrificed at a predetermined time. For each rat, 0.4 MBq in 200 μL of phosphate buffer, pH 7, was injected via a tail vein, and three animals were sacrificed 24 h postinjection. The tissues of interest were collected and weighed separately prior to counting.

Pharmacokinetics. Two mice (20 and 28 g) were anaesthetized and administered 250 μL single bolus tail vein injections containing 12 MBq of the bisphophonate **25a**. The animals were placed under a gamma camera (Argus, ADAC) equipped with a 6 mm pinhole collimator. Dynamic data were acquired using a 128 × 128 matrix size, the field of view was zoomed to the middle 25 × 25 cm of the camera, and 2 min frames were collected for 2 h.

Antitumor Efficacy Studies. A skeletal metastases model was established by injecting a total of 1×10^6 cells of the human breast carcinoma line MT-1 (1) into the left vintricula of the heart (LV) of 4−5 weeks old nude rats.[29] A primary osteogenic sarcoma model was established by intratibial (it) injection of 1×10^6 OHS cells into nude rats.[31,32] A total of 20 animals, allocated in four groups ($n = 5$ for each group) were used for both cell lines. The animals inoculated with MT-1 cells were treated with 200, 300, or 400 MBq/kg of **25a** 7 days after cell injection. The animals inoculated with OHS cells were treated with 100, 200, or 400 MBq/kg of **25a** 7 days after cell inoculation. The control groups received saline only. Doses were corrected for the actual weight of each animal and administered by single bolus tail vein injections.

Radioactivity Measurements. Radioactivity measurements of the labeled compounds were carried out with a liquid scintillation counter (Beckman LS 6500, Beckman Instruments) or with a Capintec CRC-7R radioisotope calibrator (Capintec). The radioactivity level of the excised organs was measured with an automated gamma counter (LKB Wallac 1282, Turku, Finland).

Acknowledgment. We thank Ms. S. G. Vik for skilled technical assistance. The financial support of the Norwegian Research Council is highly acknowledged.

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JM021107V