

## **Preclinical Evaluation of DO3P-AME-DO3P: A Polyazamacrocyclic Phosphonate for Diagnosis and Therapy of Skeletal Metastases**

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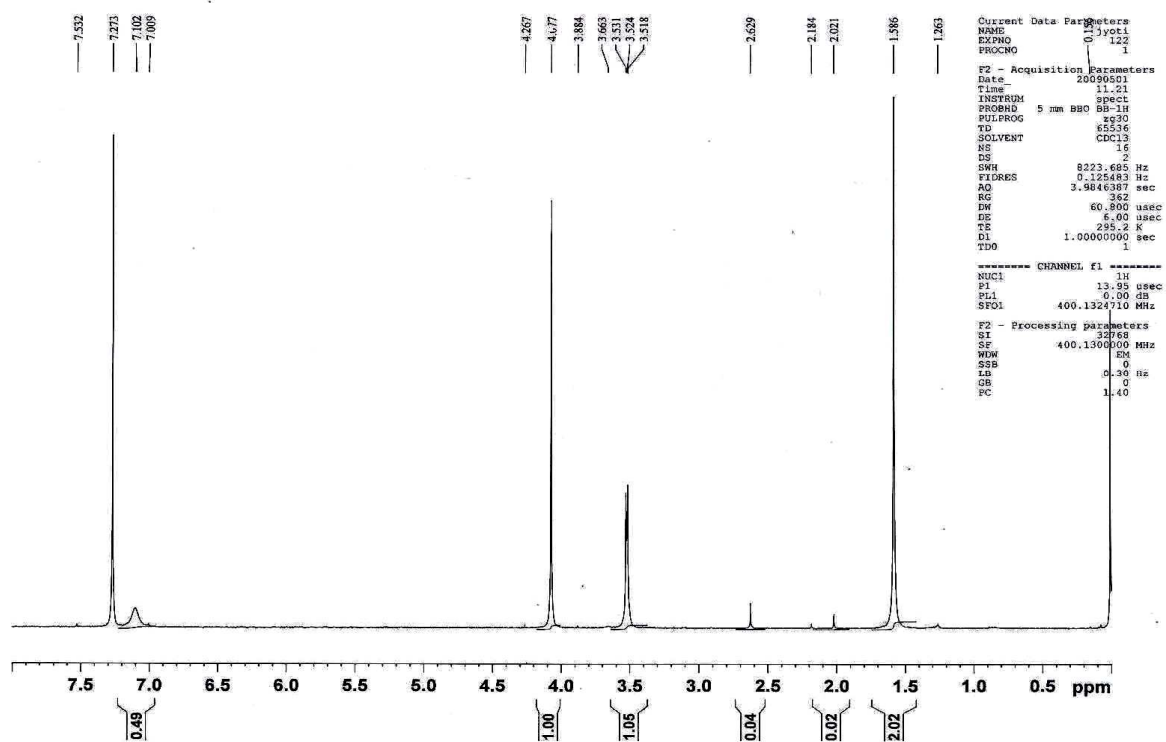


Figure S1.  $^1\text{H}$  NMR spectrum of 2-chloro-N-[2-(2-chloro-acetyl-amino)-ethyl]-acetamide (1).

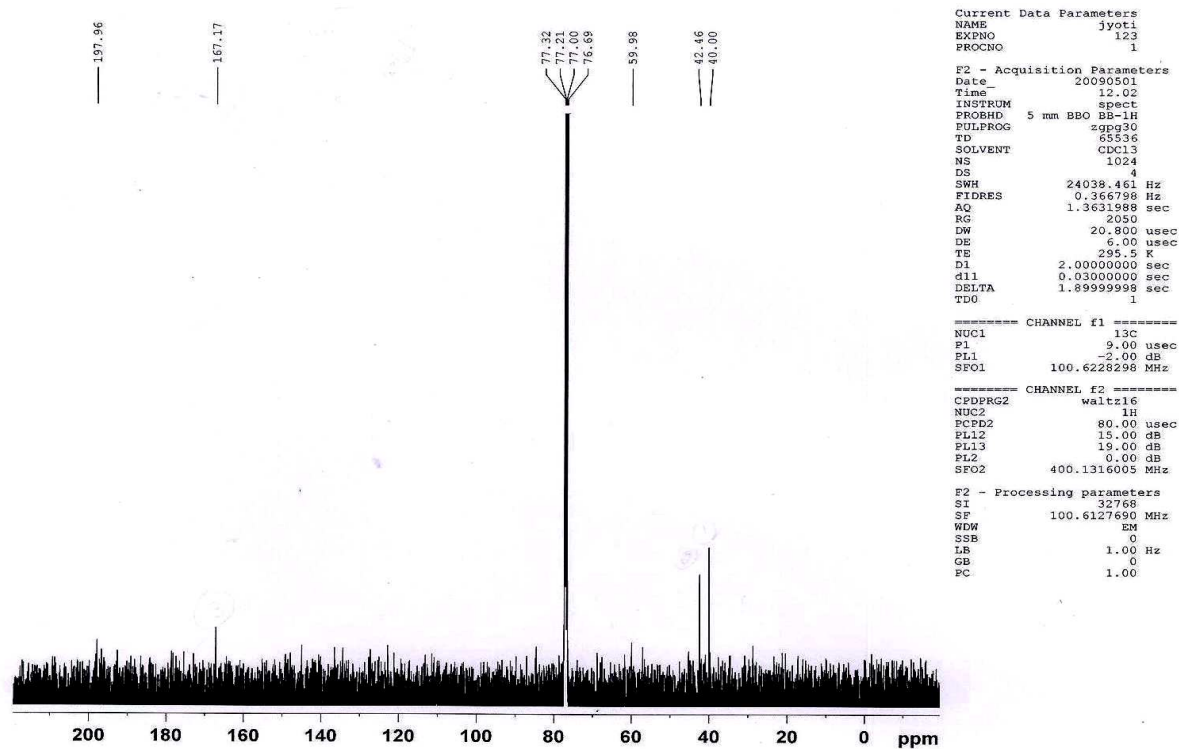


Figure S2.  $^{13}\text{C}$  NMR spectrum of 2-chloro-N-[2-(2-chloro-acetyl-amino)-ethyl]-acetamide (1).

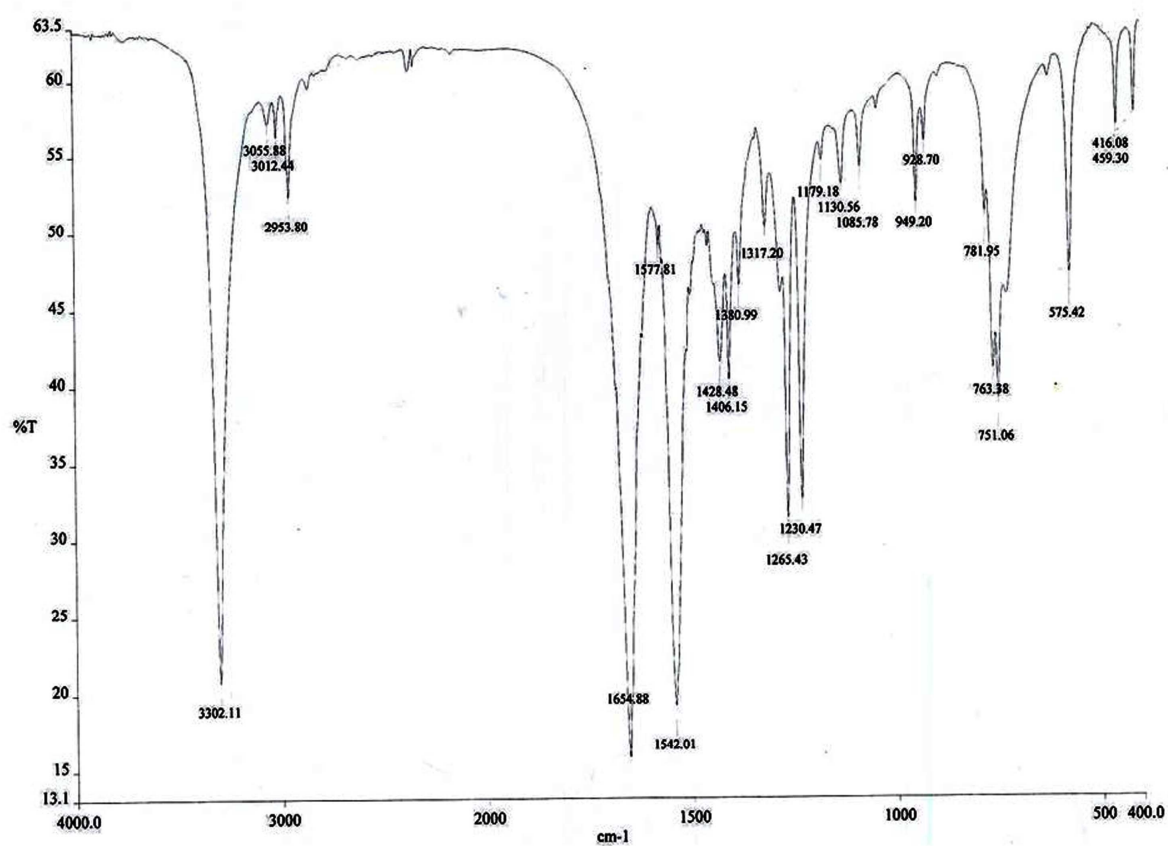


Figure S3. IR spectrum of 2-chloro-N-[2-(2-chloro-acetylamino)-ethyl]-acetamide (1).

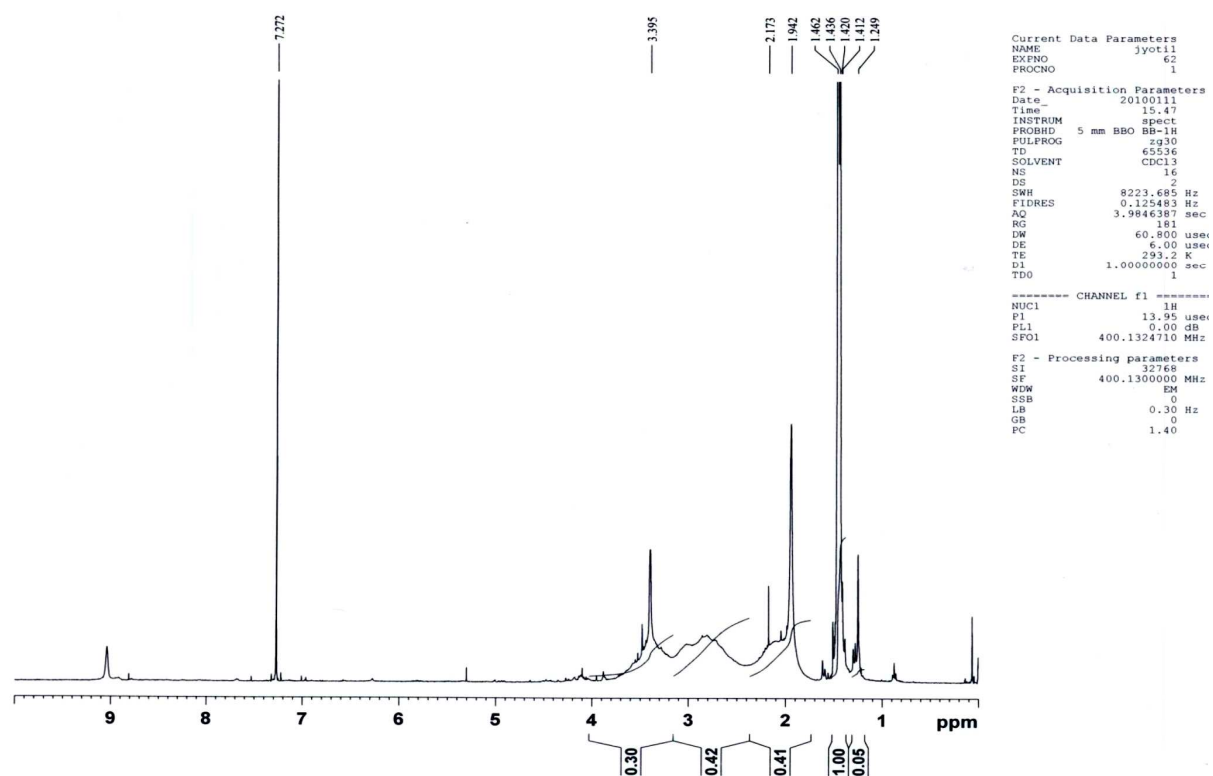


Figure S4.  $^1\text{H}$  NMR spectrum of 10'-bis(acetamido)ethane-bis[1,4,7-tri(carbobutoxymethane)]-1,4,7,10-tetraazacyclododecane (2).

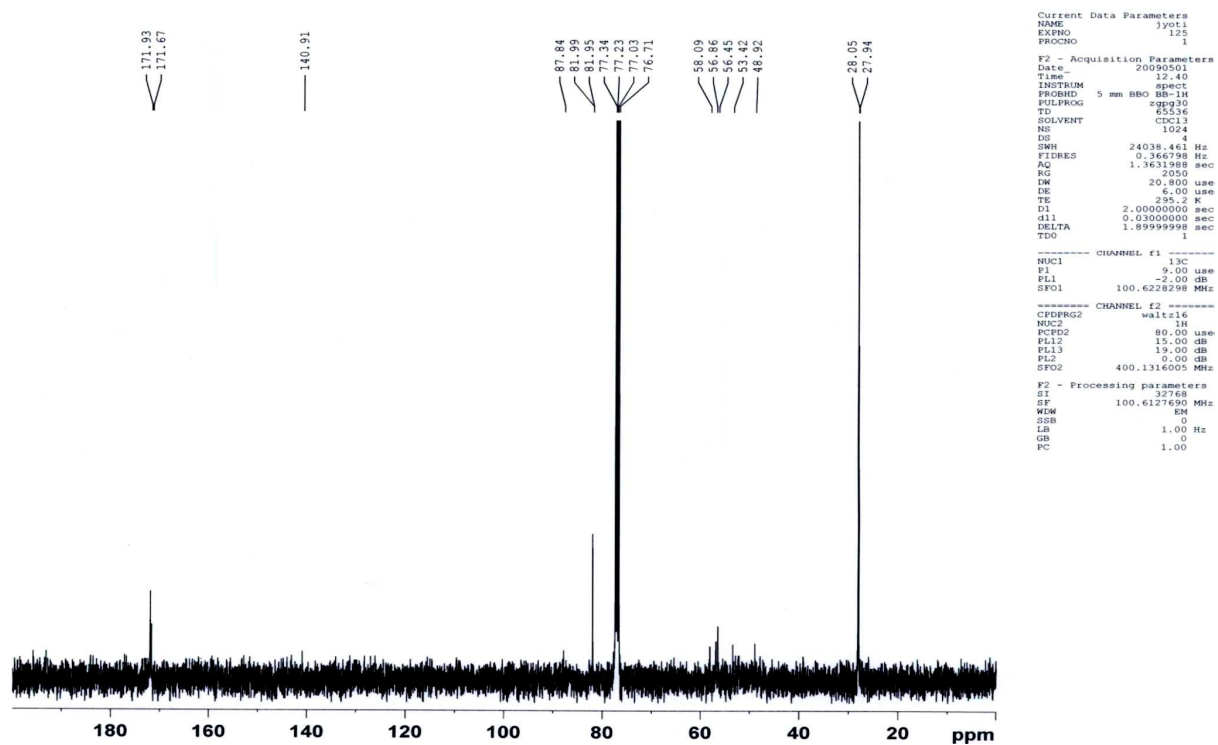


Figure S5.  $^{13}\text{C}$  NMR spectrum of 10'-bis(acetamido)ethane-bis[1,4,7-tri(carbobutoxymethane)]-1,4,7,10-tetraazacyclododecane (2).

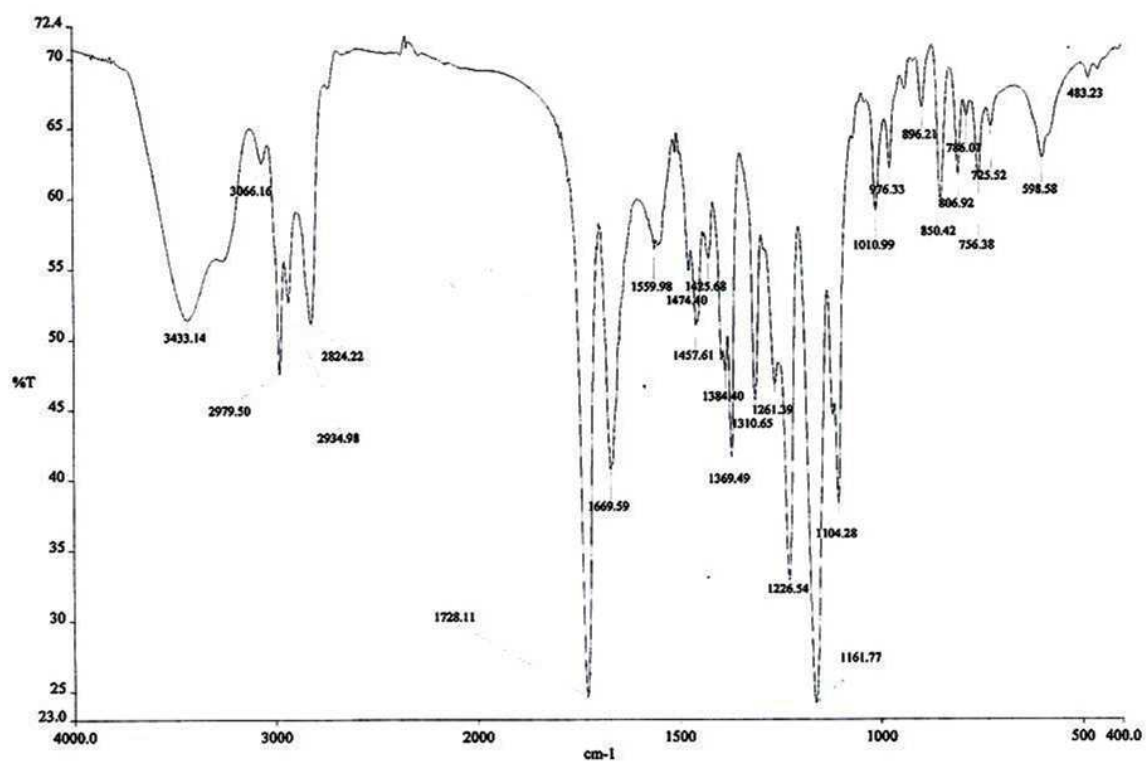


Figure S6. IR spectrum of 10'-bis(acetamido)ethane-bis[1,4,7-tri(carbobutoxymethane)]-1,4,7,10-tetraazacyclododecane (2).

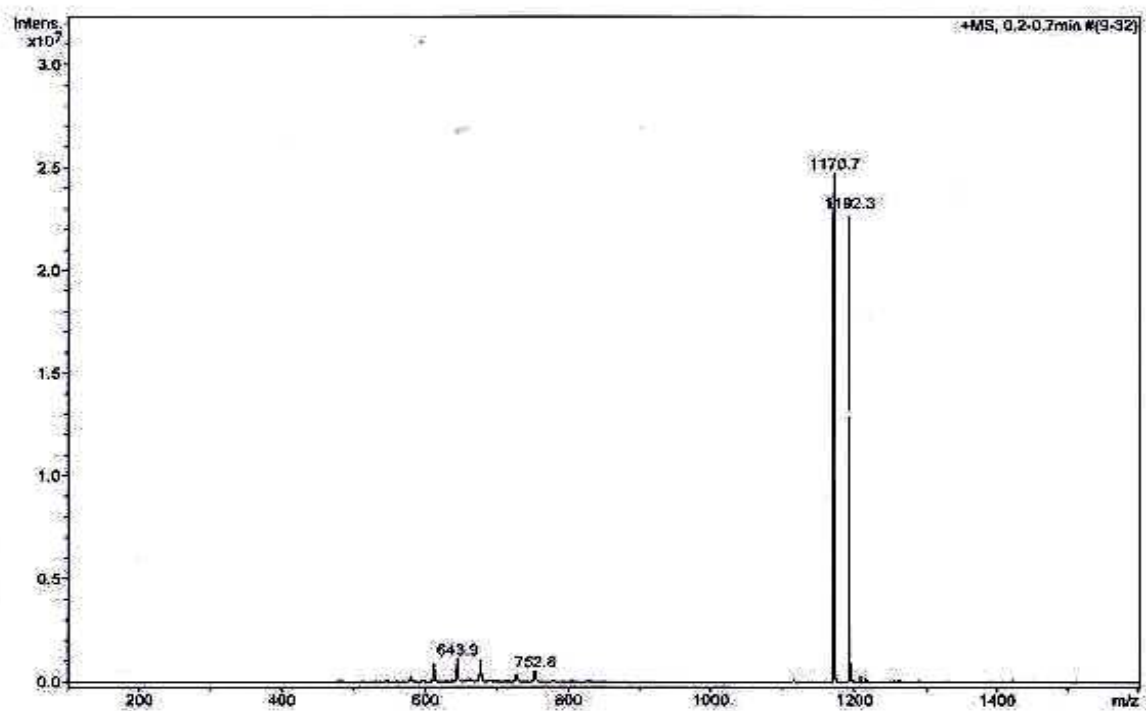


Figure S7. Mass spectrum of 10'-bis(acetamido)ethane-bis[1,4,7-tri(carbobutoxymethane)]-1,4,7,10-tetraazacyclododecane (2).

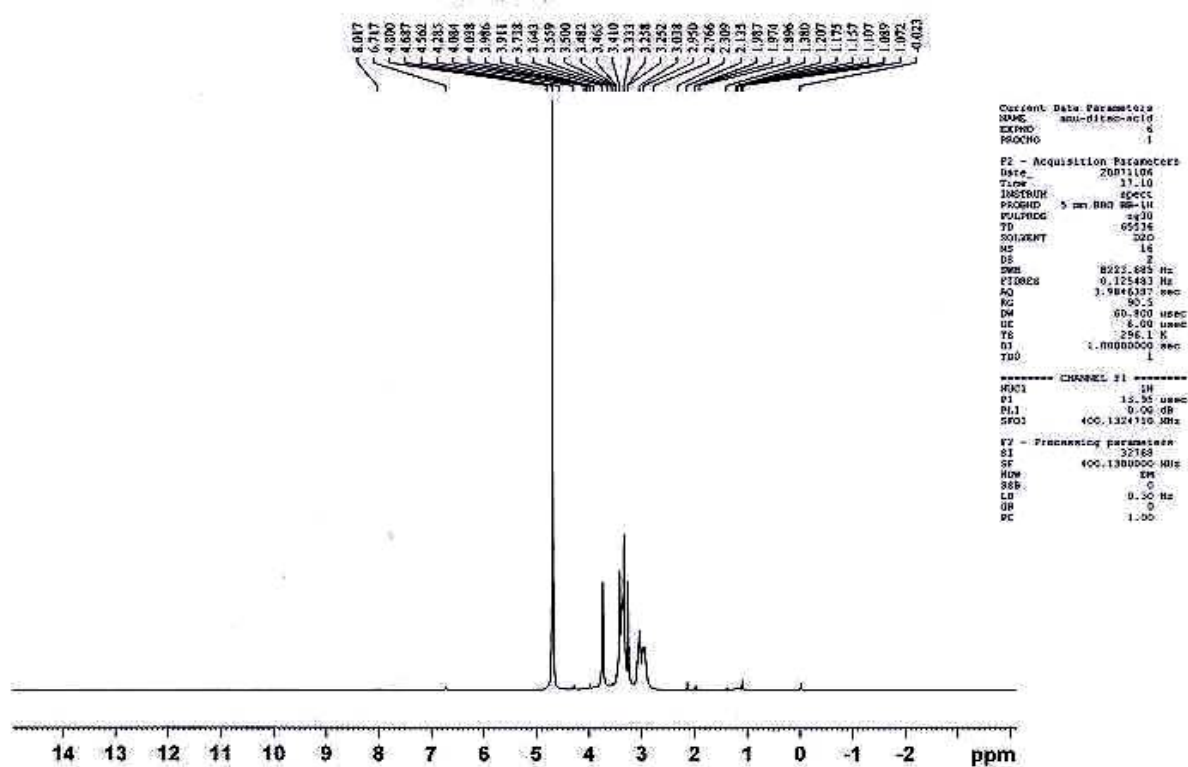


Figure S8.  $^1\text{H}$  NMR spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(carboxymethane)-1,4,7,10-tetraazacyclododecane] (DO3A-AME-DO3A).

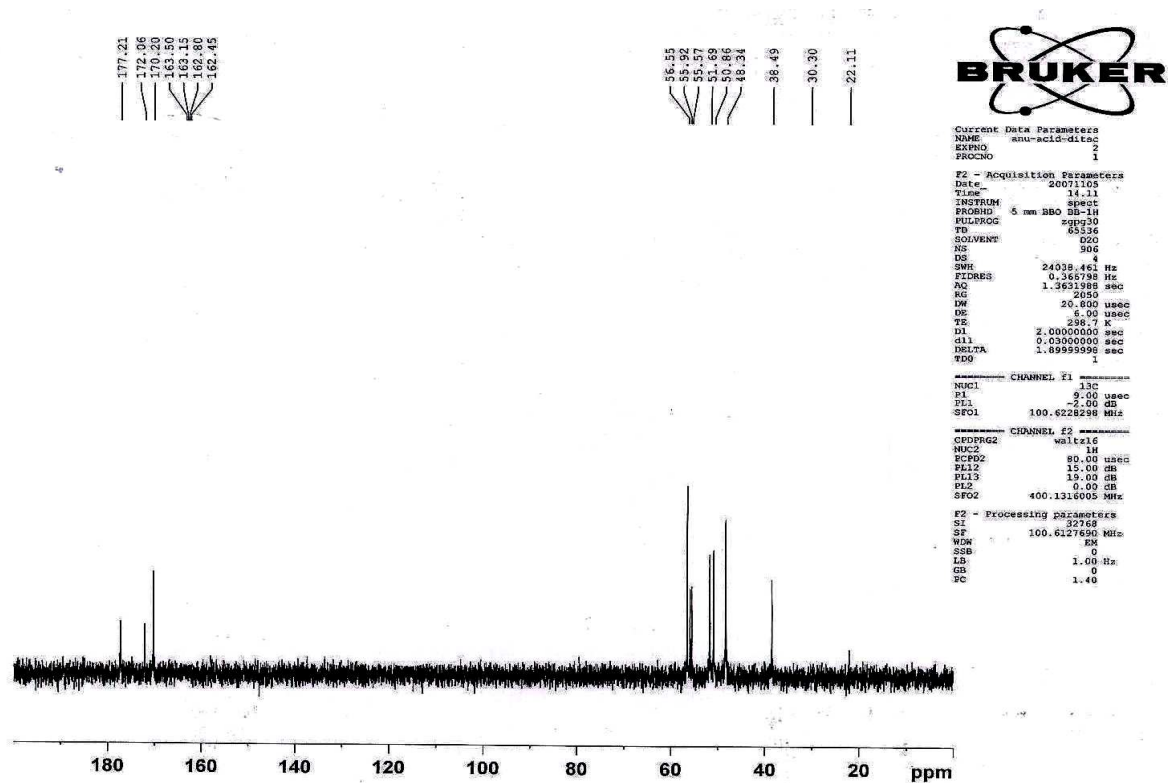


Figure S9.  $^{13}\text{C}$  NMR spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(carboxymethane)-1,4,7,10-tetraazacyclododecane] (DO3A-AME-DO3A).

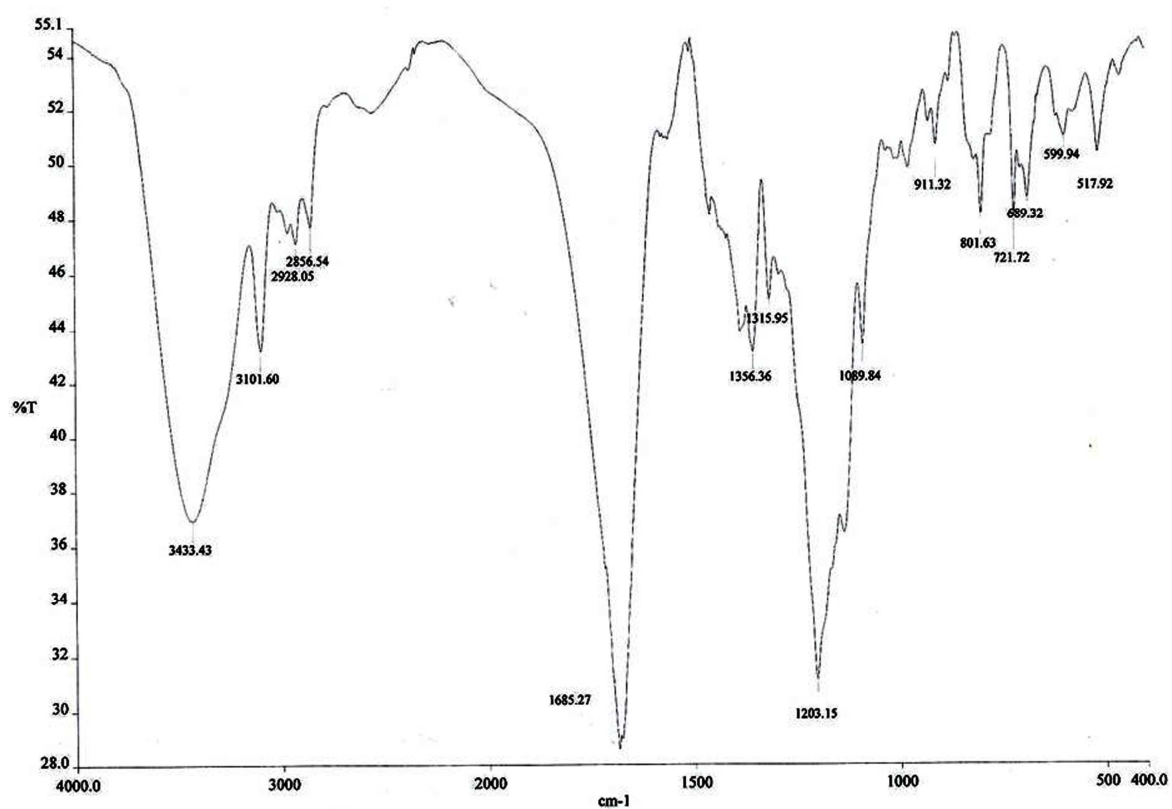


Figure S10. IR spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(carboxymethane)-1,4,7,10-tetraazacyclododecane] (DO3A-AME-DO3A).

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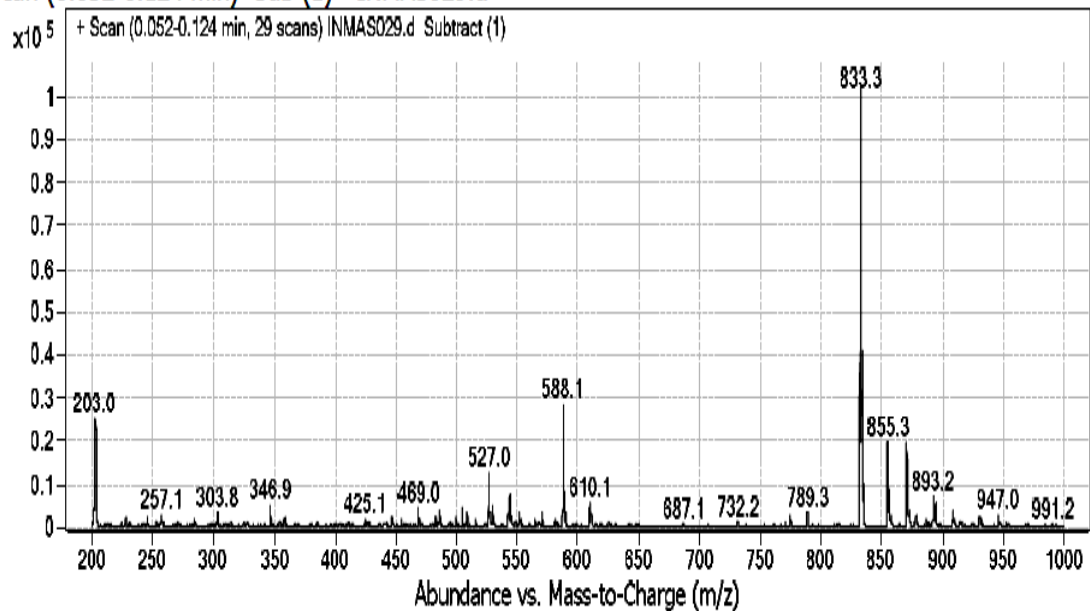


Figure S11. Mass spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(carboxymethane)-1,4,7,10-tetraazacyclododecane] (DO3A-AME-DO3A).

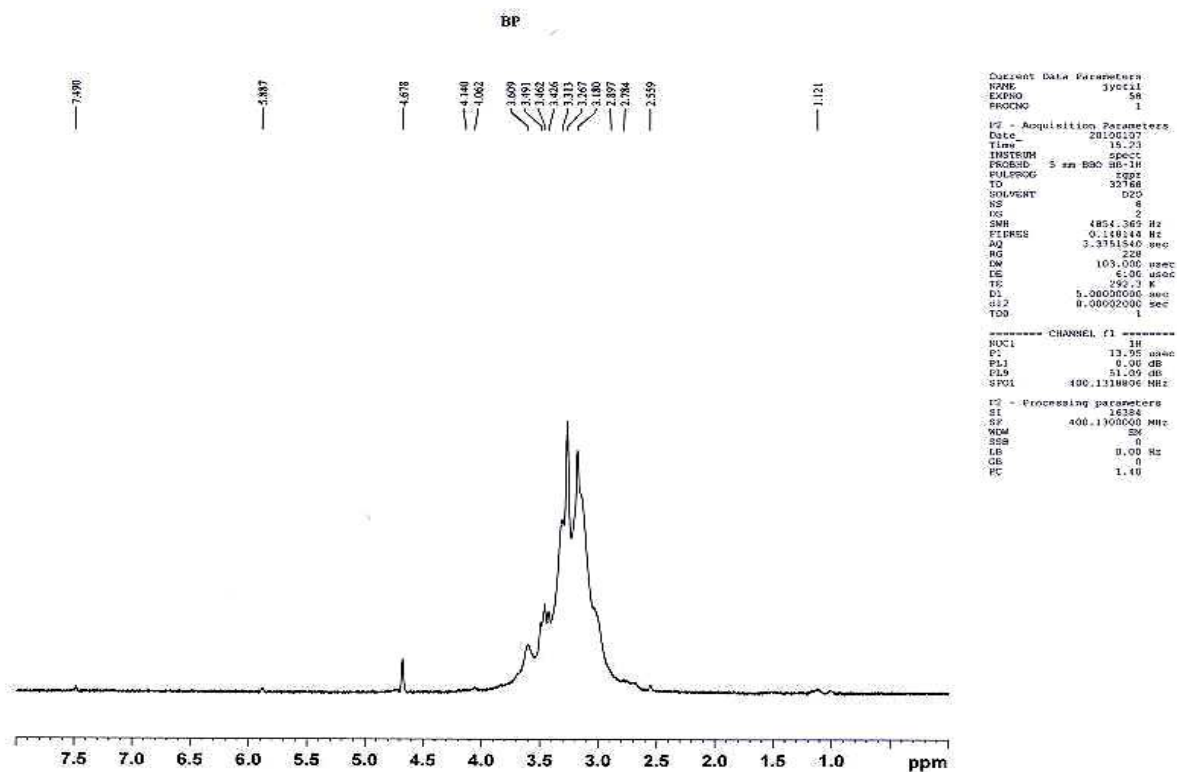


Figure S12.  $^1\text{H}$  NMR spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane] (DO3P-AME-DO3P).

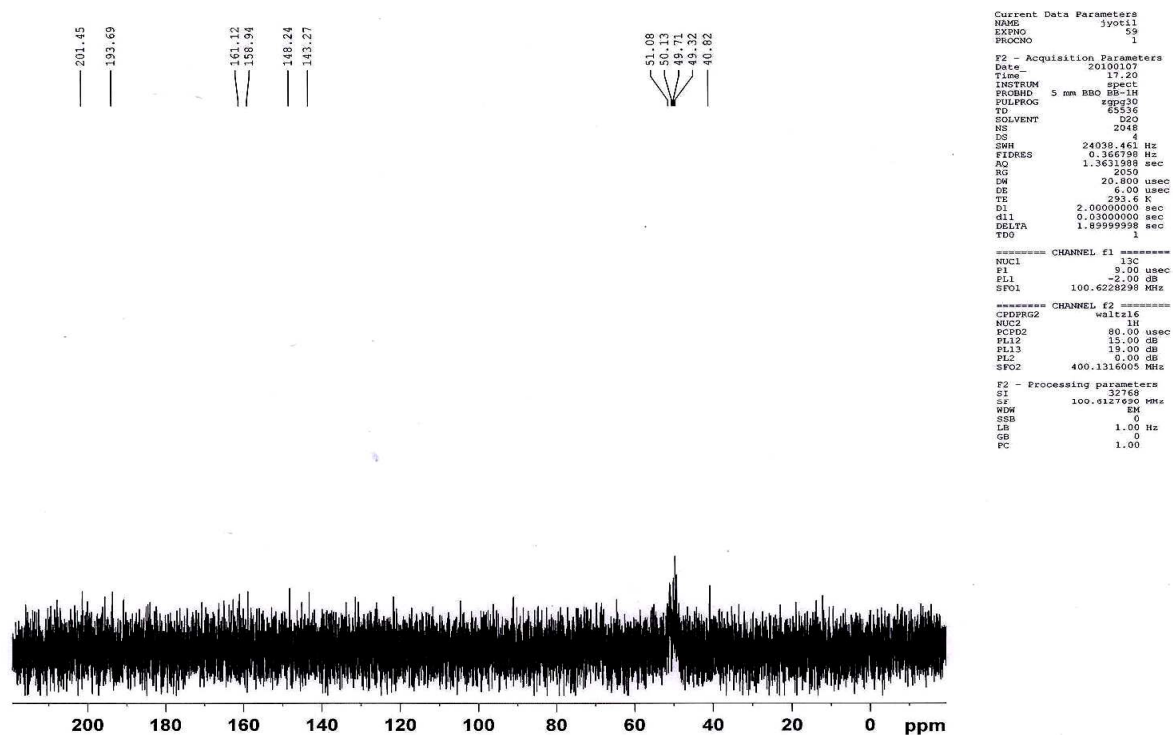


Figure S13.  $^{13}\text{C}$  NMR spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane] (DO3P-AME-DO3P).

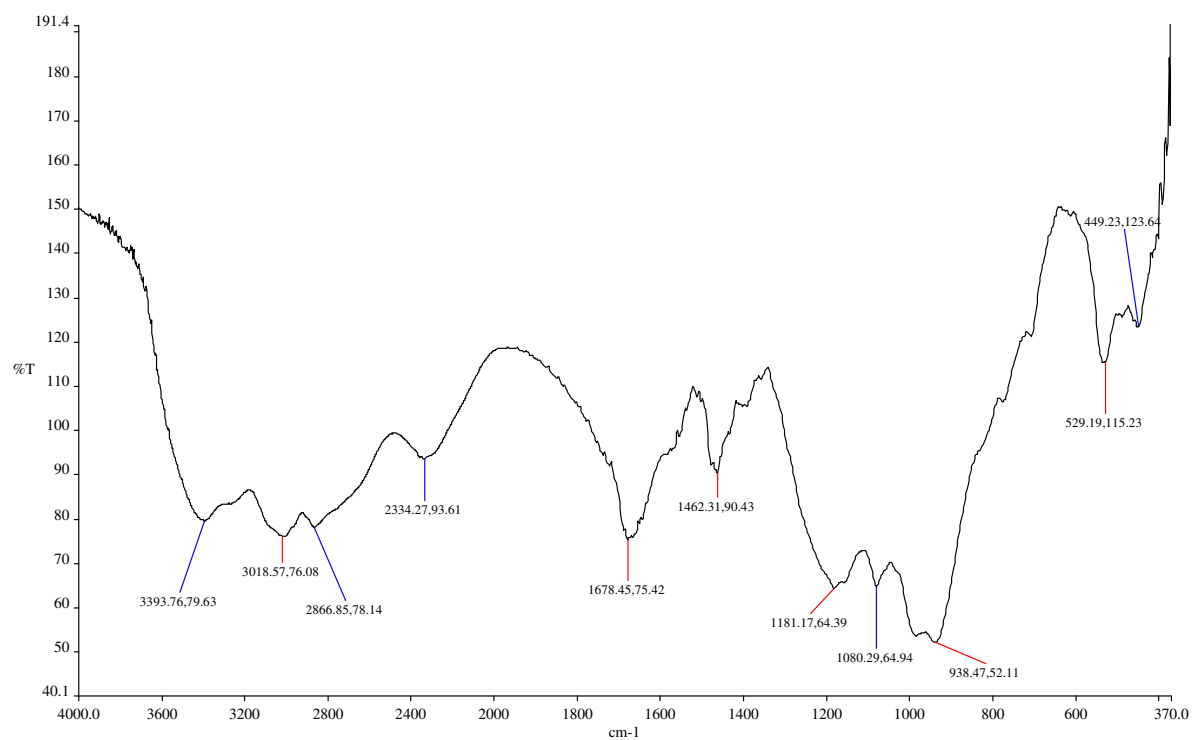


Figure S14. IR spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane] (DO3P-AME-DO3P).

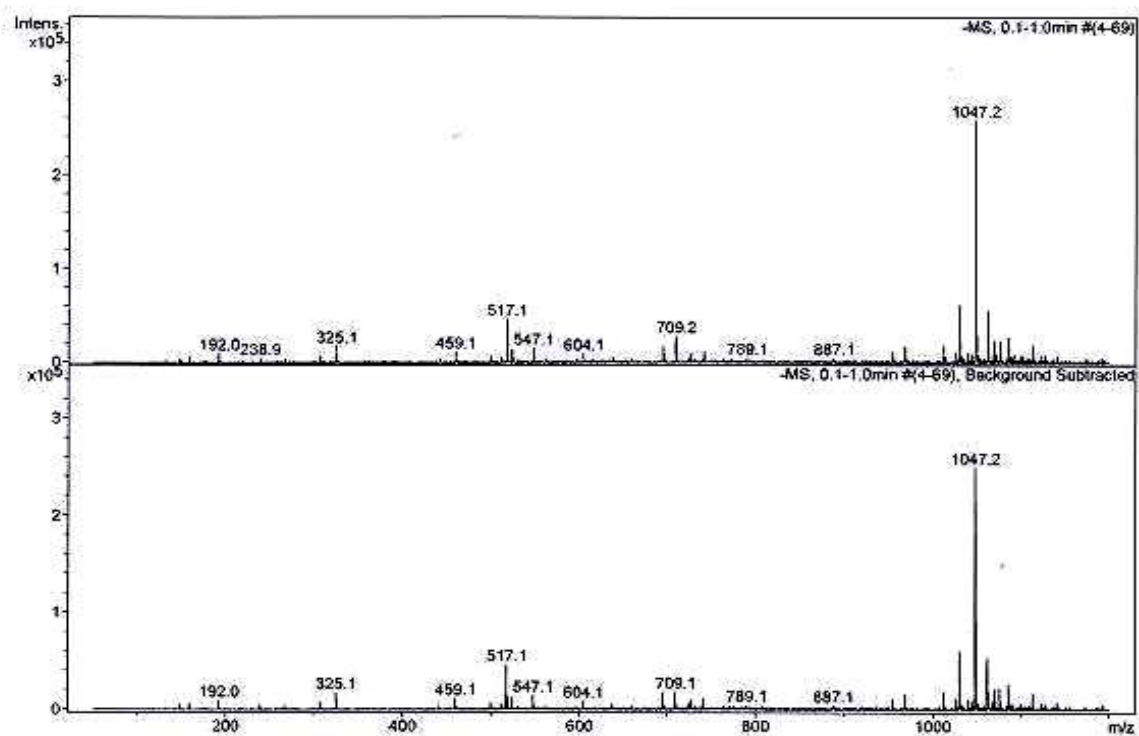


Figure S15. Mass spectrum of 10'-bis(acetamido)-ethane-bis[1,4,7-tri(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane] (DO3P-AME-DO3P).

Table S1. Biodistribution data (% ID/g) of  $^{99m}\text{Tc}$ -MDP on BALB/c mice after i.v. injection

|           | 1 h             | 2 h             | 4 h             | 24 h             |
|-----------|-----------------|-----------------|-----------------|------------------|
| Blood     | $0.86 \pm 0.16$ | $0.78 \pm 0.14$ | $0.70 \pm 0.13$ | $0.18 \pm 0.02$  |
| Heart     | $0.12 \pm 0.02$ | $0.11 \pm 0.03$ | $0.08 \pm 0.02$ | $0.02 \pm 0.001$ |
| Lungs     | $0.1 \pm 0.02$  | $0.07 \pm 0.01$ | $0.06 \pm 0.01$ | $0.02 \pm 0.002$ |
| Liver     | $1.2 \pm 0.23$  | $0.92 \pm 0.24$ | $0.83 \pm 0.19$ | $0.27 \pm 0.11$  |
| Spleen    | $0.13 \pm 0.03$ | $0.12 \pm 0.03$ | $0.12 \pm 0.03$ | $0.04 \pm 0.01$  |
| Kidney    | $7.7 \pm 1.12$  | $7.2 \pm 1.41$  | $6.36 \pm 1.01$ | $2.1 \pm 0.17$   |
| Stomach   | $0.62 \pm 0.16$ | $0.6 \pm 0.17$  | $0.51 \pm 0.13$ | $0.18 \pm 0.09$  |
| Intestine | $0.63 \pm 0.17$ | $0.56 \pm 0.18$ | $0.42 \pm 0.17$ | $0.16 \pm 0.04$  |
| Muscle    | $0.31 \pm 0.09$ | $0.27 \pm 0.08$ | $0.18 \pm 0.05$ | $0.06 \pm 0.01$  |
| Bone      | $4.2 \pm 0.82$  | $4.8 \pm 0.78$  | $5.21 \pm 0.91$ | $3.87 \pm 0.32$  |

Table S2. Biodistribution data (% ID/g) of  $^{99m}\text{Tc}$ -DMS on BALB/c mice after i.v. injection

|           | 1 h              | 2 h              | 4 h             | 24 h             |
|-----------|------------------|------------------|-----------------|------------------|
| Blood     | $0.78 \pm 0.14$  | $0.71 \pm 0.12$  | $0.60 \pm 0.11$ | $0.38 \pm 0.05$  |
| Heart     | $0.13 \pm 0.02$  | $0.11 \pm 0.01$  | $0.08 \pm 0.01$ | $0.04 \pm 0.001$ |
| Lungs     | $0.09 \pm 0.009$ | $0.07 \pm 0.007$ | $0.05 \pm 0.01$ | $0.03 \pm 0.002$ |
| Liver     | $1.1 \pm 0.30$   | $1.0 \pm 0.26$   | $0.89 \pm 0.29$ | $0.47 \pm 0.07$  |
| Spleen    | $0.11 \pm 0.01$  | $0.10 \pm 0.009$ | $0.08 \pm 0.05$ | $0.03 \pm 0.004$ |
| Kidney    | $6.9 \pm 1.03$   | $6.5 \pm 0.9$    | $6.34 \pm 1.11$ | $3.1 \pm 0.10$   |
| Stomach   | $0.60 \pm 0.57$  | $0.52 \pm 0.49$  | $0.48 \pm 0.12$ | $0.28 \pm 0.04$  |
| Intestine | $0.28 \pm 0.63$  | $0.25 \pm 0.55$  | $0.21 \pm 0.17$ | $0.17 \pm 0.05$  |
| Muscle    | $0.28 \pm 0.07$  | $0.27 \pm 0.08$  | $0.18 \pm 0.05$ | $0.06 \pm 0.01$  |
| Bone      | $3.1 \pm 0.76$   | $7.2 \pm 0.91$   | $5.01 \pm 0.81$ | $3.27 \pm 0.22$  |