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Effective Methods for the Biotinylation of Azamacrocycles

Sara J. Krivickas,[†] Emiliano Tamanini,[†] Matthew H. Todd,*[,‡] and Michael Watkinson*[,†]

School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London, E1 4NS, United Kingdom, and School of Chemistry, University of Sydney, New South Wales 2006, Australia

m.todd@chem.usyd.edu.au; m.watkinson@qmul.ac.uk

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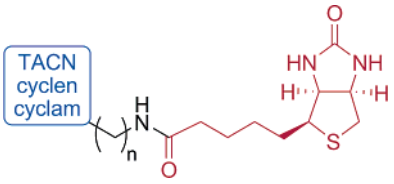

The biotin-(strept)avidin interaction remains a gold standard of model biological recognition events. The biotinylation of azamacrocycles permits the investigation of signal transduction between this recognition event and the metal center of an azamacrocycle complex, of wide potential interest in biosensing. There are no generally applicable procedures in the literature for such functionalizations. We report here a comprehensive investigation into the attachment of biotin to TACN, cyclen, and cyclam. Effective methods have been found for each ring. The efficacy of the functionalization is critically dependent on the nature of the azamacrocycle.

Introduction

Azamacrocylic ligands continue to receive considerable attention, and their metal complexes find widespread utility as a consequence of their robust and well-defined coordination chemistry.[1] This broad ligand class is perhaps most commonly associated with applications in the general area of radiopharmaceuticals[2] but has also been applied in many other areas such as catalysis,[3] biomimetics,[4] and sensor devices.[5]

We recently became interested in the regioselective N-functionalization of azamacrocycles with biologically relevant recognition motifs with the aim of utilizing the metal coordinated to the macrocycle to sense a subsequent binding event. We elected to employ the biotin-(strept)avidin system as our model because of its well-established high binding affinity[6] and the fact that a number of biotinylated metal complexes has recently been shown to bind to (strept)avidin,[7] indicating that the presence of a cationic center does not affect the binding unduly.

Exploration of the metal's ability to report such binding requires methodology for the synthesis of biotinylated azamacrocycles. Archibald et al.[8] have recently reported an elegant strategy for the biotinylation of a cross-bridged cyclam derivative, although binding of the biotinylated copper complex to (strept)avidin was not demonstrated. Moreover, as we were keen to establish the possibility of using generic synthetic methods

[†] University of London.
[‡] University of Sydney.

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(7) For recent examples, see: (a) Skander, M.; Humbert, N.; Collot, J.; Gradinaru, J.; Klein, G.; Loosli, A.; Sauser, J.; Zocchi, A.; Gilardoni, F.; Ward, T. *J. Am. Chem. Soc.* **2004**, *126*, 14411−14418. (b) Eckermann, A. L.; Barker, K. D.; Hartings, M. R.; Ratner, M. A.; Meade, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 11880−11881. (c) Lo, K. K-W.; Hui, W-K. *Inorg. Chem.* **2005**, *44*, 1992−2002. (d) Letonder, C.; Pordea, A.; Humbert, N.; Ivanova, A.; Mazurek, S.; Novic, M.; Ward, T. R. *J. Am. Chem. Soc.* **2006**, *128*, 8320−8328.

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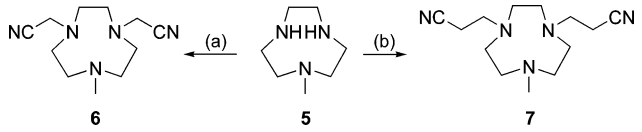
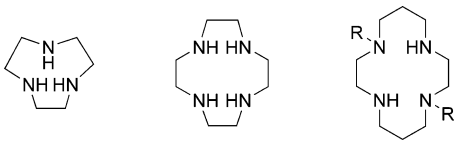
Published on Web 09/29/2007

SCHEME 1[a]



[a] Reagents and conditions: (a) $ClCH_2CN$, K_2CO_3, MeCN, Δ, 24 h, N_2 and (b) acrylonitrile, Δ, N_2.

for the N-functionalization of a range of azamacrocycles, we did not view this as a viable approach. Several strategies toward our target ligands could be conceived, using the array of reported methods for the functionalization of azamacrocycles. However, it quickly became clear that it would not be possible to develop generic methods as each azamacrocycle displays synthetic idiosyncrasies and the synthetic strategy employed must be tailored to the individual azamacrocycle. Herein, we report our findings in the functionalization of TACN, **1**, cyclen, **2**, dimethyl cyclam, **3**, and cyclam, **4**; of the azamacrocycles studied, cyclam has proven to be the easiest to derivatize with biotin.

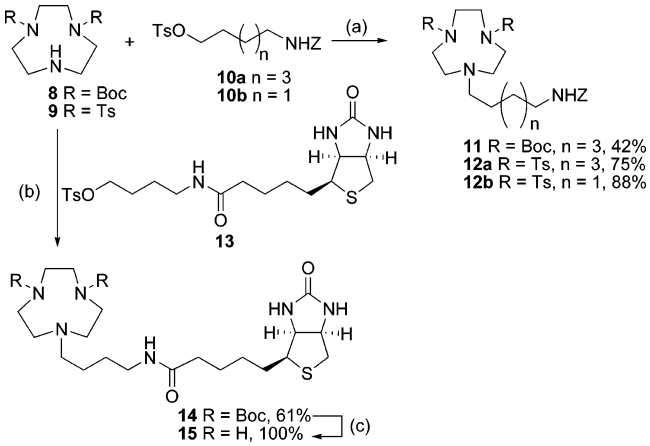


Results and Discussion

Alkylation of TACN. Our first attempts to functionalize an azamacrocyclic ligand with biotin centered on **1**. Our aim was to find a means of introducing a primary amine group to the macrocycle via N-alkylation to which we could then couple the carboxylic acid of biotin. We also wished to have sufficient synthetic flexibility to allow the spacer unit between the primary amine and the azamacrocycle to be varied to investigate whether or not the spacer influenced the effect the binding of avidin had upon the metal center. Thus, Schröder's procedure for the N-alkylation of **5** with electrophilic nitriles appeared to be a particularly attractive strategy (Scheme 1),[9] as this gave us a means of introducing both primary amine and carboxylic acid functional groups that could be used in a variety of ways to incorporate biotin; indeed, if an unsymmetrical coupling could be effected, the remaining amine or carboxylic acid could be further utilized (e.g., to mount the material on a solid support or surface). The synthesis of **6** and **7** from **5** proceeded smoothly, although we found that **6** formed in higher yield when K_2CO_3 was used as the base rather than the reported triethylamine. Nitrile hydrolysis could be easily achieved, but in our hands, nitrile reduction proved capricious, so we decided to investigate alternative strategies for the introduction of amines. We thus turned to the di-Boc-protected analogue **8** (Scheme 2), which we were able to prepare using the reported methods.[10]

Benniston et al. have recently utilized **8** in a series of peptide couplings to attach pendant arms to the 1,4,7-triazacyclononane framework via amide linkages, and a number of Zn(II) complexes of such amide-containing azamacrocycles have been

SCHEME 2[a]



[a] Reagents and conditions: (a) K_2CO_3, MeCN, Δ, 24 h, N_2; (b) **13**, K_2CO_3, MeCN, Δ; and (c) 20% TFA, DCM.

reported.[10−13] We were, however, concerned that this methodology would not be suitable for all of the ligands in which we are interested. Our first concern with using an amide linker was that Lewis acid activated amide hydrolysis could occur. While Alsfasser has reported that such amide linkers are stable in buffered solution at pH 6.8, hydrolysis was found to occur in neutral solution and at higher pHs.[14] Thus, if we were to apply these macrocyclic conjugates in vivo, the loss of the biological receptor was a real danger; these concerns were subsequently justified (vide infra). Our other concern with an amide linker centered on the unpredictable nature of its interaction with a metal center. Not only are structurally authenticated Werner complexes of tertiary amides extremely rare,[15,16] but the mode of the tertiary amide nitrogen−metal interaction is difficult to predict, even in closely related systems,[16,17] which could reduce the stability and/or change the properties of the coordination complex, thus limiting our ability to detect a binding event of our macrocycle−biotin conjugate in a predictable and reproducible manner. We thus sought to introduce hydrolytically robust alkyl pendant arms. α-,ω-Bromoamines have been utilized in related systems as a means of introducing a primary amine pendant arm by simple nucleophilic substitution of the alkyl bromide when the primary amine is Z-protected.[18] We preferred to utilize the sulfonate esters **10** instead in the first instance and found that the target macrocycle **11** could be isolated in moderate yield (Scheme 2). We also found that sulfonamide protection of the macrocyclic amine nitrogen atoms, as in **9**, significantly improved yields, suggesting that carbamate protecting groups for the azamacrocycle are less compatible with this

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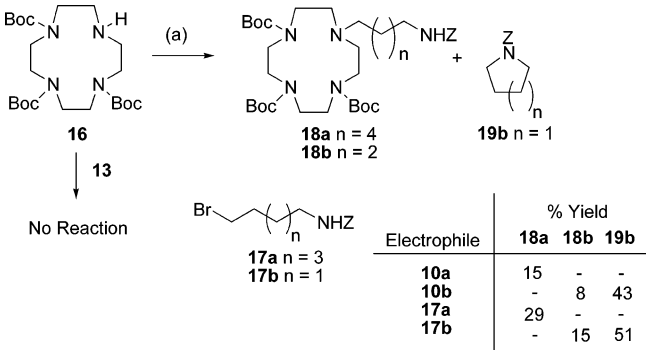
SCHEME 3[a]



Electrophile	% Yield 18a	18b	19b
10a	15	-	-
10b	-	8	43
17a	29	-	-
17b	-	15	51

[a] Reagents and conditions: (a) **10** or **17**, K_2CO_3, MeCN, Δ, 4 days.

chemistry. The use of **9** instead of **8** introduces other issues associated with sulfonamide deprotection, which we felt would be problematic in the presence of biologically relevant recognition motifs. Therefore, we attempted to effect the global deprotection of **11** using conventional methods[19] and then couple the product to biotin in the hope that the increased reactivity of the primary amine (derived from the Z-carbamate in **11**) might give some regiocontrol. Unfortunately, intractable mixtures resulted.

In an attempt to avoid this problem, we decided to try to incorporate biotin in the electrophile prior to the coupling reaction. Gratifyingly, reaction of **8** with **13** (see Supporting Information for the synthesis of **13**) proceeded smoothly in 61% yield to give the biotinylated ligand **14**. Removal of the Boc groups could easily be effected with TFA to give the free amine **15** as its TFA salt quantitatively.

Alkylation of Cyclen. Confident that we had established an effective method for the biotinylation of **1**, we attempted to apply the same conditions to the biotinylation of **2**. It quickly became clear that finding a generic functionalization strategy was unlikely to be achieved. Although tri-Boc-protected cyclen **16** could be reliably prepared following literature procedures,[20] its reaction with **13** under the same conditions that had led to the formation of **14** consistently resulted in the quantitative reisolation of starting materials (Scheme 3). We then investigated the efficacy of electrophiles **10** in the alkylation of **16** and again saw a distinct difference in behavior between the different macrocyclic ligands. This time, the sulfonate esters gave a very poor yield of the target macrocycles **18** with either starting materials recovered (**10a**, 56% recovered) or significant quantities of cyclic carbamate **19b** (from **10b**) being formed. We therefore investigated the efficacy to α-,ω-bromides **17**, which although superior to **10**, were still unsatisfactory. We attribute this to the alkylation of **16** being much slower than it is for **8**. There is no obvious explanation for this other than that **16** adopts a conformation that retards its alkylation. We elected not to investigate the efficacy of sulfonamide protection of the macrocyclic nitrogen atoms in this case given the perennial problems already encountered with the removal of this group.

As an alternative strategy, we decided to utilize reductive aminations that have previously been reported to occur with **16** by Chae et al.[3] While the reported reductive amination between **16** and **20c**[21] proceeded smoothly to give **21c** in 77% yield, our attempt to extend the length of the chain by using **20a** saw a dramatic reduction in the yield of **18a** to 64% (Scheme 4). Furthermore, we were unable to prepare **20b** ($n = 2$) due to competing intramolecular cyclization of the aldehyde. Nonetheless, the primary amine in **22** could be readily revealed by hydrogenation under standard conditions,[19] and the primary amine effectively coupled to biotin using HATU,[22] a reagent that we have generally found to be the best agent for the coupling of biotin to azamacrocycles (vide infra). The free amine, **24**, was isolated as its TFA salt by simple deprotection of **23**.[19]

Acylation of Cyclen. We turned to the use of peptide couplings for the functionalization of cyclen, despite our concerns over amide stability in the presence of Lewis acids, as our efforts to systematically vary the length of the spacer between the macrocycle and the biotin had met with somewhat limited success. We were keen to utilize Fmoc-protected amino acids in the coupling reactions so that the methodology could ultimately be applied to the development of solid phase combinatorial libraries of azamacrocycles derivatized with short oligopeptides.[23] Unfortunately, adaptation of an effective procedure for the coupling of Z-protected glycine to **16**[10] was unsuccessful for the Fmoc-protected glycine, **25**. Fortunately, adaptation of the procedure reported by Ritter et al.[11] proceeded smoothly for a number of Fmoc-protected amino acids, giving the desired amides in good to excellent yield (**28** = 86% and **29** = 64%) (Scheme 5).

We were readily able to remove the Fmoc and Boc protecting groups[19] yielding **34**−**36** and to show that a complex of amide **34**, prepared from copper(II) chloride in aqueous solution buffered at pH 9.8, appeared to be stable in methanolic solution, as judged by a combination of IR and UV−vis spectroscopies and ES MS. Encouraged, we therefore elaborated the synthesis further and found that biotin could be coupled to **28**, following Fmoc group removal, in good yield to ultimately give the TFA salt of **38**. The biotinylated macrocycle could be metalated in the same manner as **34**, which to the best of our knowledge, represents only the second example of a metal complex of a biotinylated azamacrocyclic ligand[8] (see Supporting Information). Unfortunately, closer investigation of the coordination chemistry of **34**−**36** caused us some concern as the coordination mode of the ligand appeared to be critically dependent on the anion used. With chloride, clear evidence of a shift in the carbonyl band from 1678 to 1589 cm^{-1} was observed, consistent with amide oxygen coordination, as reported by Alsfasser for related cyclam complexes;[16] however, a second carbonyl band was also observed at 1686 cm^{-1} (cf. 1678 cm^{-1} in the free ligand), which is indicative of the presence of another amide mode (i.e., the presence of different coordination modes of the ligand). Furthermore, when we investigated the use of other anions, we found that the perchlorate complex appeared to be unstable in methanolic solution, with changes occurring in its UV−vis spectrum after only a few hours at room temperature. Given that the ultimate application we envisage for the functionalized metal complexes in sensors requires both well-defined and robust coordination chemistry, we feel that this

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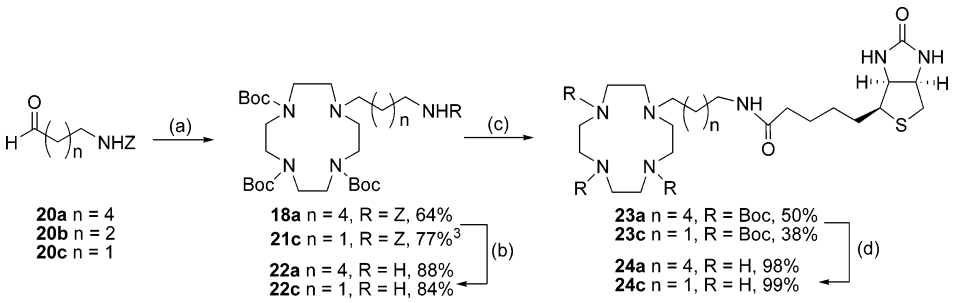
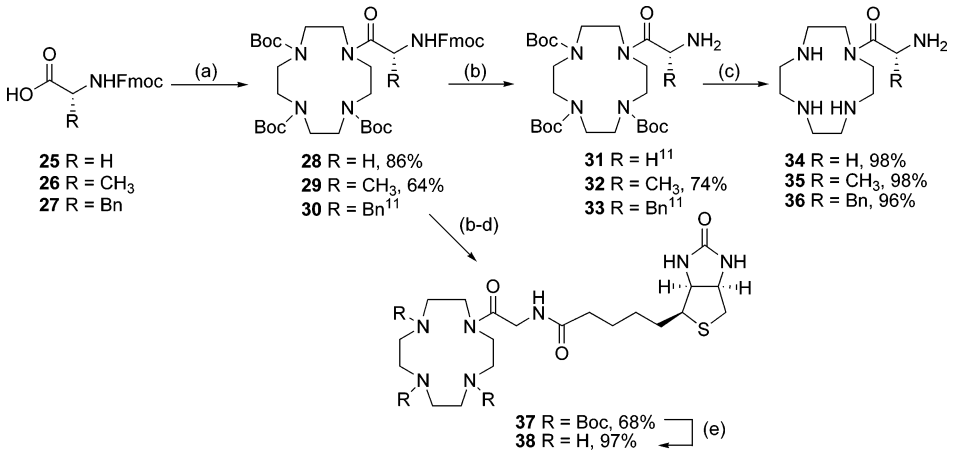
SCHEME 4[a]



[a] Reagents and conditions: (a) **16**, $NaBH(OAc)_3$, THF; (b) Pd/C, H_2, EtOH; (c) HATU, HOBT, DMAP, biotin, DMF/DCM; and (d) 20% TFA, DCM.

SCHEME 5[a]



[a] Reagents and conditions: (a) **16**, HATU, HOBt, DMAP, DCM/DMF, 40 °C, 2 days; (b) TBAF, MeCN; (c) 20% TFA, DCM; (d) biotin, HATU, HOBt, DCM/DMF, 40 °C, 2 days; and (e) 20% TFA, DCM.

behavior, while interesting, will preclude the use of this linker in the devices.

Acylation of Cyclam. As discussed, cyclam−amide conjugates are known to undergo Lewis acid-catalyzed hydrolysis, and we have noted that the solution stability of some of the cyclen amide complexes is also poor. Nevertheless, we felt it worthwhile to investigate whether the methods shown in Scheme 5 could also be applied to cyclam. To our surprise, the Fmoc-amino acid-protected coupling strategy that had been so effective for **16**[11] proved to be ineffective for the equivalent cyclam, **39**, with the poor-yielding couplings that occurred further hampered by complex reaction mixtures being formed. We therefore turned to using Z-protected glycine **40**,[10] which gave **41** in quantitative yield using DCC as the coupling agent. Simple palladium-catalyzed hydrogenation[19] gave the required coupling precursor, **42** (Scheme 6). We then found that the efficient coupling of biotin to **42** was particularly sensitive to the coupling agent used, with HATU being by far the most effective. Our efforts to effect couplings of biotin using other methods were consistently lower yielding (e.g., HBTU/DIPEA gave **43** in only 30% yield). Deprotection of **43** was again easily achieved with TFA to give **44**, again as its TFA salt. We then attempted to introduce copper(II) into the cyclam ring by carefully neutralizing the TFA salt of **44** in a methanol/water mixture to pH 8 with 1 N NaOH prior to the addition of $Cu(ClO_4)_2 \cdot 6H_2O$. Unfortunately, inexpedient hydrolytic cleavage of the amide occurred, which was confirmed by the formation of crystals of $[Cu(\mathbf{4})(ClO_4)_2]$. We have also observed amide cleavage with simpler groups attached to the ring nitrogen (e.g., single amino acids).[24]

Alkylation of Cyclam. The hydrolytic instability of these cyclam conjugates led us to investigate the alkylation of cyclam. We returned to the reductive amination strategy described previously for cyclen. Again, the reductive amination of **39** with **20c** proceeded smoothly (Scheme 7). Elaboration of the macrocyclic scaffold in the usual way lead to biotinylated cyclam **48c** in acceptable yield, and we were able to prepare an authenticated copper(II) complex of it and demonstrate its binding to avidin.[6] In contrast, the same methodology was completely ineffective for **3** with the reduced aldehyde being recovered quantitatively. Interestingly, the reactivity of **3** generally appears to be lower than that of the other macrocycles investigated. For example, its alkylation with **10** and **17** was ineffective, and only its alkylation with the highly reactive acrylonitrile proceeded in satisfactory yield.[9]

In view of our inability to effectively vary the linker length in **48c** between the amine and the macrocycle using the reductive amination strategy, we turned to the use of ethyl α-bromoethyl acetate, which has been used extensively in the functionalization of cyclen derivatized macrocycles,[25] but which appears to have been little utilized in cyclam chemistry. Our initial reluctance to explore this methodology lay in the necessity to elaborate the carboxylic acid in **49** with a singly protected diamine such

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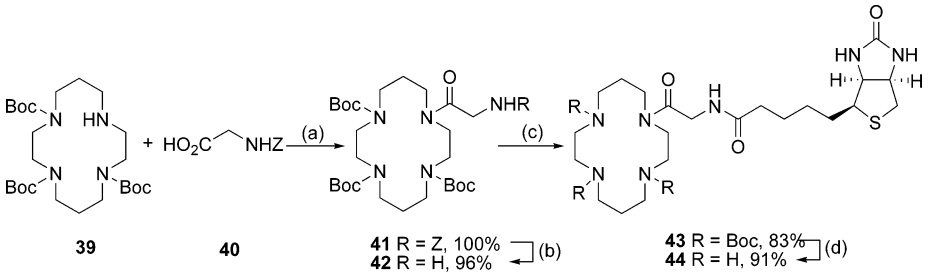
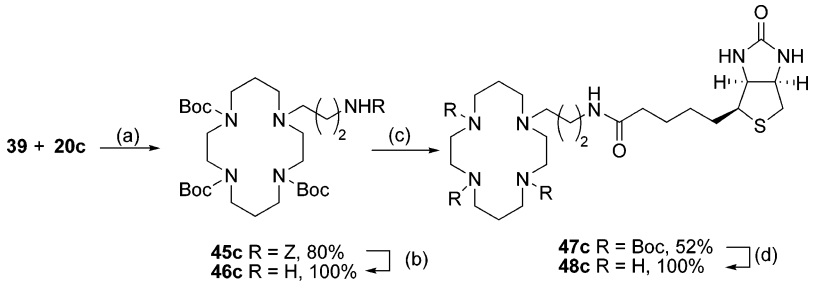
SCHEME 6[a]



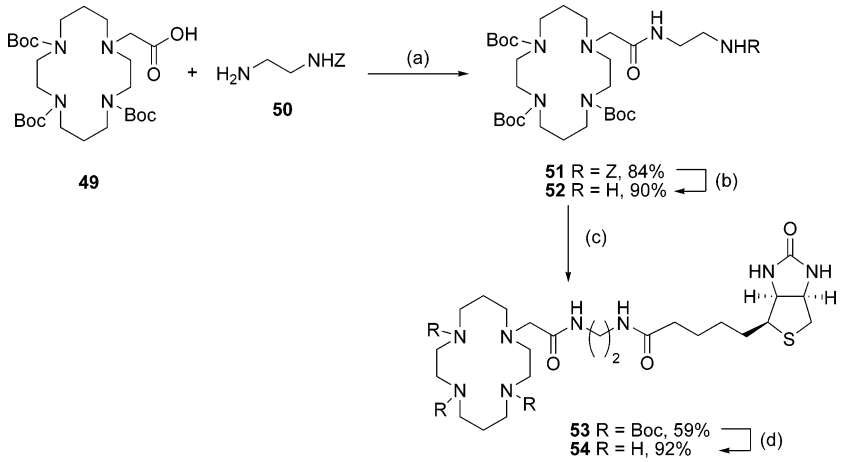
[a] Reagents and conditions: (a) DCC, DMAP, DCM; (b) Pd/C, H_2, MeOH; (c) HATU, DIPEA, DMAP, biotin, DMF; and (d) 20% TFA, DCM.

SCHEME 7[a]



[a] Reagents and conditions: (a) $NaBH(OAc)_3$; (b) Pd/C, H_2, MeOH; (c) HATU, DIPEA, DMAP, biotin, DMF; and (d) 20% TFA, DCM.

SCHEME 8[a]



[a] Reagents and conditions: (a) DCC, DMAP, DCM; (b) Pd/C, H_2, MeOH; (c) HATU, DIPEA, DMAP, biotin, DMF; and (d) 20% TFA.

as **50**. While ethylene diamine can be readily desymmetrized, this is a more difficult procedure for longer diamines.[19] In addition, we felt that this method added unnecessary protection/deprotection steps. Despite these reservations, the required carboxylic acid was readily prepared by alkylation of **39** with ethyl α-bromoethyl acetate, which was then saponified. The carboxylic acid in **49** was effectively coupled to amine **50** with deprotection of the Z-carbamate proceeding smoothly (Scheme 8). Coupling of **52** to biotin was again found to proceed smoothly using HATU to give **53**, which was readily converted to **54** in the usual way.

Conclusion

A broad spectrum of methods has been investigated for the selective functionalization of azamacrocycles with biotin. Although we have found that there is apparently no generic method that can be applied to all of the macrocycles investigated, we have been able to find effective routes to biotinylate **1**, **2**, and **4**; in contrast, **3** has proven to be considerably less reactive. It is difficult, prima facie, to rationalize these observations, and further studies are required into the conformational preferences of these protected macrocycles. Reports relating to the variation in reactivity in such systems are sparse, and we have been unable to find any directly relevant analogues. Sherry and Kovacs have exploited the variation in pK_a of azamacrocyclic ligands to effect a variety of selective functionalization strategies.[26,27] Presumably, this is a conformational effect, and this has been exploited in host−guest chemistry where variations in anion binding of azamacrocycles has been directly related to pK_a.[28] Moreover, it has been noted that the effectiveness of polyazamacrocycle

(26) Kovacs, Z.; Sherry, A. D. *Synthesis* **1996**, 759−763.
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alkylation is dependent on the size of the ligand.[29] Finally, Alder et al. have correlated hydrogen bonding in bridged aminal analogues with conformational effects.[30] In contrast to the present study, in none of these examples were the nitrogen atoms of the macrocycles protected as Boc-carbamates, and thus, a direct analogy can only be speculative, and investigations to this end are currently ongoing. Most of the successful methods we describe allow some variation in the nature of the macrocycle-biotin linker so that fine-tuning of the effect that (strept)-avidin binding has on the metal center might be achieved. In addition, many of the intermediates provide a means by which other biological markers might be introduced to the macrocyclic framework.

Experimental Section

The following compounds were prepared according to the reported procedures: **5**−**7**,[9] **8**,[10] **9**,[9] **10a**,[18] **10b**,[25] *N*-benzyloxycarbonyl-4-amino-1-butanol, *N*-benzyloxycarbonyl-6-aminohexanol, **17**,[18] **16**,[10] **20c**,[21] and **39**.[31] For compounds that have been previously reported but were prepared by different methods, see the Supporting Information.

7-(6-Benzyloxycarbonylamino-hexyl)-[1,4,7]triazonane-1,4-dicarboxylic Acid Di-*t*-butyl Ester (11). To a stirred solution of **8** (14 mg, 0.043 mmol) and K_2CO_3 (12 mg, 0.085 mmol) in dry acetonitrile (5 mL) was added **10a** (26 mg, 0.064 mmol), and the solution was refluxed overnight under a nitrogen atmosphere. The reaction was cooled, acetonitrile was removed in vacuo, the residue was taken up in dichloromethane, washed with water, dried over $MgSO_4$, and the volatiles were removed in vacuo. The crude residue was purified by silica gel chromatography (1:1, ethyl acetate/petroleum spirits) to afford **11** as a colorless oil (10 mg, 42%): ν_{max} (CH_2Cl_2)/cm^{-1} 1685; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38−7.28 (m, 5H), 5.05 (s, 2H), 4.75 (bs, 1H), 3.52−3.40 (m, 4H), 3.30−3.14 (m, 6H), 2.66−2.55 (m, 4H), 2.51−2.41 (m, 2H), 1.66−1.21 (m, 30H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.4, 154.6, 154.4, 135.7, 127.5, 127.1, 65.5, 55.8, 53.2, 53.1, 52.9, 52.6, 50.0, 49.62, 49.55, 49.3, 48.6, 40.0, 28.7, 27.6, 26.8, 26.0, 25.7; MS (ESI) *m*/*z*: 563.5 [(M + H)$^+$], 455.4, 429.4, 330.3; HRMS (ES) calcd for $C_{30}H_{51}N_4O_6$ [(M + H)$^+$] 563.3803, found 563.3801.

{6-[4,7-Bis-(toluene-4-sulfonyl)-[1,4,7]triazonan-1-yl]-hexyl}-carbamic Acid Benzyl Ester (12a). To a stirred solution of **9** (200 mg, 0.46 mmol) and K_2CO_3 (130 mg, 0.92 mmol) in dry acetonitrile (10 mL) was added **10a** (220 mg, 0.55 mmol), and the solution was refluxed overnight under a nitrogen atmosphere. The reaction was cooled, acetonitrile was removed in vacuo, the residue was taken up in dichloromethane, washed with water, and dried over $MgSO_4$, and the volatiles were removed. The crude residue was purified by silica gel chromatography (10:1, dichloromethane/methanol) to afford **12a** as a colorless oil (242 mg, 75%): ν_{max} (CH_2Cl_2)/cm^{-1} 3391, 2931, 2858, 1717, 1597, 1527, 1454; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 7.66 (d, *J* = 8.4 Hz, 4H), 7.39−7.22 (m, 9H), 5.08 (s, 2H), 4.85 (bs, 1H), 3.55−3.44 (m, 4H), 3.24−3.05 (m, 6H), 2.91−2.79 (m, 4H), 2.52 (t, *J* = 7.0 Hz, 2H), 2.37 (s, 6H), 1.58−1.20 (m, 8H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 156.6, 143.5, 136.8, 135.4, 129.8, 128.5, 128.1, 127.3, 66.6, 57.4, 55.9, 52.6, 51.5, 41.1, 29.9, 27.7, 27.1, 26.7, 21.7, 21.4; MS (ESI) *m*/*z*: 671 [(M + H)$^+$], 141, 90; HRMS (ESI) calcd for $C_{34}H_{47}N_4O_6S_2$ [(M + H)$^+$] 671.2932, found 671.2937.

{4-[4,7-Bis-(toluene-4-sulfonyl)-[1,4,7]triazonan-1-yl]-butyl}-carbamic Acid Benzyl Ester (12b). Compound **12b** was made according to the previous procedure for **11**. Compound **9** (500 mg, 1.14 mmol), **10b** (560 mg, 1.37 mmol), and K_2CO_3 (316 mg, 2.29 mmol) in dry acetonitrile (20 mL) afforded **12b** as a colorless oil (646 mg, 88%): ν_{max} (CH_2Cl_2)/cm^{-1} 3403, 2936, 1716, 1643, 1597, 1524; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 7.65 (d, *J* = 8.2 Hz, 4H), 7.38−7.22 (m, 9H), 5.14−5.01 (m, 3H), 3.50−3.39 (m, 4H), 3.28−3.12 (m, 6H), 2.91−2.79 (m, 4H), 2.56 (t, *J* = 6.3 Hz, 2H), 2.43(s, 6H), 1.65−1.41 (m, 4H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 156.6, 143.6, 136.8, 135.3, 129.9, 128.5, 128.0, 127.2, 66.5, 56.7, 55.7, 52.9, 51.7, 27.5, 25.1, 21.6; MS (ESI) *m*/*z*: 666 [(M + Na)$^+$], 643 [(M + H)$^+$]; HRMS (ESI) calcd for $C_{32}H_{43}N_4O_6S_2$ [(M + H)$^+$] 643.2619, found 643.2620.

Toluene-4-sulfonic Acid 4-[5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoylamino]-butyl Ester (13). A stirred suspension of **C** (see Supporting Information; 120 mg, 0.382 mmol), *p*-toluene sulfonyl chloride (146 mg, 0.742 mmol), triethylamine (0.12 mL, 0.841 mmol), and DMAP (5 mg, 0.038 mmol) in dichloromethane (5 mL) was stirred for 3 days at room temperature. The solvent was removed in vacuo, and the crude material was purified by silica gel column chromatography (1:10 methanol/dichloromethane) to give **13** (31 mg, 20%) as a pale yellow oil: ν_{max}(Nujol)/cm^{-1} 1666; ^{1}H NMR (CD_3OD, 270 MHz): δ = 7.78 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 2H), 4.48 (dd, *J* = 7.7, 4.7 Hz, 1H), 4.29 (dd, *J* = 7.9, 4.5 Hz, 1H), 4.04 (t, *J* = 6.2 Hz, 2H), 3.24−3.15 (m, 1H), 3.12(t, *J* = 4.4 Hz, 2H), 2.92 (dd, *J* = 12.9, 4.9 Hz, 1H), 2.69 (d, *J* = 12.9 Hz, 1H), 2.45 (s, 3H), 2.17 (t, *J* = 7.2 Hz, 2H), 1.79−1.32 (m, 10H); ^{13}C NMR (CD_3OD, 67.5 MHz): δ = 174.7, 164.8, 145.2, 133.2, 129.6, 127.8, 70.3, 62.0, 60.3, 55.7, 39.7, 38.2, 35.5, 28.4, 28.2, 26.1, 25.5, 25.2, 20.3; MS (ESI) *m*/*z*: 492 [(M + Na)$^+$], 470 [(M + H)$^+$], 298, 226; HRMS (ESI) calcd for $C_{21}H_{32}N_3O_5S_2$ [(M + H)$^+$] 470.1778, found 470.1780.

7-{4-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-6-yl)-pentanoylamino]-butyl}-[1,4,7]triazonane-1,4-dicarboxylic Acid Di-*t*-butyl Ester (14). A solution of **8** (31 mg, 0.094 mmol), **13** (52 mg, 0.114 mmol), and K_2CO_3 (35 mg, 0.251 mmol) in anhydrous acetonitrile (5 mL) was refluxed under a nitrogen atmosphere for 4 days. After cooling, the solvent was removed in vacuo, and the crude material was purified by silica gel column chromatography (10% methanol/dichloromethane) to give **14** (36 mg, 61%) as a colorless oil: ν_{max}(CH_2Cl_2)/cm^{-1} 1686, 1555, 1461, 1416, 1366; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 6.62−6.46 (bm, 1H, NH), 6.14−5.98 (bm, 1H, NH), 4.56−4.46 (m, 1H), 4.36−4.26 (m, 1H), 3.52−3.10 (m, 11H), 2.91 (dd, *J* = 12.6, 4.9 Hz, 1H), 2.74 (d, *J* = 12.9 Hz, 1H), 2.66−2.42 (m, 6H), 2.28−2.14 (m, 2H), 2.05−1.58 (m, 6H), 1.54−1.34 (m, 22H); ^{13}C NMR, not reported due to the presence of rotamers, it was not possible to interpret the spectrum; MS (ESI) *m*/*z*: 649.5 [(M + Na)$^+$], 627.5 [(M + H)$^+$], 528.4, 527.4, 428.4, 427.4; HRMS (ESI) calcd for $C_{30}H_{54}N_6O_6S$ [(M + H)$^+$] 627.3898, found 627.3888.

5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoic Acid (4-[1,4,7]Triazonan-1-yl-butyl)-amide-dihydrate-trifluoroacetate (15). To a solution of **14** (10 mg, 16 μmol) in DCM (1 mL), TFA (42 equiv, 6.2 mmol, 50 μL) was added, and the resulting solution was stirred at room temperature overnight. The solvent was then evaporated in vacuo to give **15** as a pale yellow solid: ν_{max}(neat)/cm^{-1} 1688, 1455. MS (ESI) *m*/*z*: 427.4 [(M + H)$^+$]. HRMS (ESI) calcd for $C_{20}H_{38}N_6O_2S$ [(M + H)$^+$], 427.2850, found 427.2858.

10-(6-Benzyloxycarbonylamino-hexyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (18a). A stirred solution of **16** (180 mg, 0.381 mmol), K_2CO_3 (105 mg, 0.763 mmol), and **10a** (248 mg, 0.611 mmol) in dry acetonitrile (10 mL) was refluxed overnight under a nitrogen atmosphere. The solution was allowed to cool, and the acetonitrile was removed in vacuo. The residue was taken up in dichloromethane, washed with water, and dried over $MgSO_4$, and the volatiles were removed. The crude material was purified by column chromatography (silica gel, 1:1 ethyl acetate/petroleum spirits) to give **18a** (39 mg, 15%) as a colorless oil: ν_{max}(CH_2Cl_2)/cm^{-1} 1686, 1460, 1416; ^{1}H NMR (270

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MHz, $CDCl_3$): δ = 7.47−7.29 (m, 5H), 5.05 (s, 2H), 4.84 (bs, 1H), 3.61−3.11 (m, 14H), 2.71−2.44 (m, 6H), 1.60−1.15 (m, 35H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 156.5, 156.2, 155.8, 155.5, 136.7, 128.6, 128.2, 79.6, 79.4, 66.7, 54.9, 53.7, 52.3, 50.1, 48.0, 47.7, 41.1, 30.0, 28.8, 28.6, 27.5, 26.7, 23.7; MS (ESI) *m*/*z*: 706 [(M + H)$^+$], 473; HRMS (ESI) calcd for $C_{37}H_{64}N_5O_8$ [(M + H)$^+$] 706.4749, found 706.4745.

10-(6-Benzyloxycarbonylamino-hexyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (18a). Alternative procedure: a solution of **16** (187 mg, 0.396 mmol), triacetoxy sodium borohydride (191 mg, 0.902 mmol), and **20a** (235 mg, 0.451 mmol) in THF (10 mL) was stirred overnight at room temperature. The solution was basified with saturated $NaHCO_3$, the volatiles were removed in vacuo, and the aqueous layer was extracted with dichloromethane. The combined organics were dried over $MgSO_4$, and the volatiles were removed in vacuo. The crude material was purified by silica gel chromatography (1:1 ethyl acetate/petroleum spirits) to afford **18a** (177 mg, 64%) as a colorless oil.

10-(4-Benzyloxycarbonylamino-butyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (18b). A stirred solution of **16** (100 mg, 0.212 mmol), K_2CO_3 (58 mg, 0.424 mmol), and **10b** (133 mg, 0.424 mmol) in dry acetonitrile (10 mL) was refluxed overnight under a nitrogen atmosphere. The solution was allowed to cool, and the acetonitrile was removed in vacuo. The residue was taken up in dichloromethane, washed with water, and dried over $MgSO_4$, and the volatiles were removed. The crude material was purified by silica gel chromatography (1:1, ethyl acetate/petroleum spirits) to give **18b** as a colorless oil (12 mg, 8%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1662, 1538, 1456, 1416; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 7.33−7.22 (m, 5H), 5.07(s, 2H), 3.53−3.10 (m, 16H), 2.63−2.40 (m, 4H), 1.48−1.50 (m, 4H), 1.39 (s, 9H), 1.37 (s, 18H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 156.5, 156.1, 155.8, 155.4, 136.7, 128.5, 128.2, 128.1, 79.6, 79.5, 79.3, 66.6, 55.0, 53.4, 51.8, 50.0, 48.1, 47.8, 47.4, 40.9, 40.6, 28.5, 28.0, 21.1; MS (ESI) *m*/*z*: 701 [(M + Na)$^+$], 678 [(M + H)$^+$]; HRMS (ESI) calcd for $C_{35}H_{60}N_5O_8$ [(M + H)$^+$] 678.4436, found 678.4438.

6-Oxo-hexyl-carbamic Acid Benzyl Ester (20a). Oxalyl chloride (0.39 mL, 3.79 mmol) was dissolved in anhydrous dichloromethane (20 mL) under a nitrogen atmosphere and cooled to −78 °C. Dimethyl sulfoxide (0.43 mL, 7.59 mmol) was added dropwise over 5 min, and the reaction mixture was stirred at −78 °C for 10 min. *N*-Benzyloxycarbonyl-6-amino-1-hexanol (0.956 mg, 3.79 mmol) in dichloromethane (50 mL) was added slowly over 5 min. The reaction mixture was then warmed to room temperature and then cooled to −78 °C and stirred for a further 10 min. Triethylamine (2.8 mL, 18.9 mmol) was added, and the reaction again was warmed to room temperature. Water (50 mL) was added, the organic layer was separated, and the aqueous layer was extracted with dichloromethane. The combined organics were dried over $MgSO_4$ and filtered, and the volatiles were removed in vacuo. Silica gel chromatography (2:3−1:1 ethyl acetate/petroleum spirits) afforded **20a** as a colorless oil: $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1697, 1645; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 9.75 (s, 1H), 7.50−7.20 (m, 5H), 5.09 (s, 2H), 4.79 (bs, 1H), 3.21 (q, *J* = 6.4 Hz, 2H), 2.44 (t, *J* = 6.4 Hz, 2H), 1.78−1.24 (m, 6H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 202.5, 136.7, 128.6, 128.2, 66.7, 43.8, 40.9, 29.8, 26.3, 21.7; MS (ESI) *m*/*z*: 250 [M + H$^+$]$^+$, 232 [M + MeOH]$^+$, 267 [M + NH_4^+]$^+$.

10-(3-Benzyloxycarbonylamino-propyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (21c). Macrocycle **21c** was prepared in an analogous manner to **18a**: **16** (258 mg, 0.547 mmol), triacetoxy sodium borohydride (397 mg, 1.873 mmol), and **20c** (180 mg, 0.938 mmol) in THF (10 mL) afforded **21c** (274 mg, 77%): $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1686, 1533, 1460, 1416; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.39−7.23 (m, 5H), 5.70 (bs, 1H), 5.05 (s, 2H), 3.64−3.05 (m, 14H), 2.70−2.41 (m, 6H), 1.70−1.52 (m, 2H), 1.41 (s, 9H), 1.38 (s, 18H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 156.72, 156.3, 155.8, 155.4, 136.9, 128.4, 128.1, 79.7, 79.5, 66.4, 55.2, 54.4, 49.9, 48.9, 47.6, 38.9, 28.7, 28.5; MS (ESI) *m*/*z*: 664[(M + H)$^+$]; HRMS (ESI) calcd for $C_{34}H_{58}N_5O_8$ [(M + H)$^+$] 664.4280, found 664.4280.

10-(3-Amino-hexyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (22a). A solution of **21a** (384 mg, 0.56 mmol), Pd/C (17 mg), and acetic acid (0.04 mL, 0.61 mmol) in methanol (7 mL) under a H_2 atmosphere was stirred overnight at room temperature. The solution was filtered, and the solvent was removed under reduced pressure to give **22a** as a colorless oil (272 mg, 88%); $\nu_{max}(CHCl_3)/cm^{-1}$ 1686; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 3.54−3.06 (m, 12H), 2.75−2.30 (m, 8H), 1.64−1.09 (m, 35H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 156.1, 155.7, 155.4, 79.4, 79.2, 55.0, 53.7, 52.6, 50.0, 48.0, 47.6, 42.2, 28.7, 28.5, 27.7, 26.9; MS (ESI) *m*/*z*: 572[(M + H)$^+$]; HRMS (ESI) calcd for $C_{29}H_{58}N_5O_6$ [(M + H)$^+$] 572.4382, found 572.4378.

10-{6-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoylamino]-hexyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (23a). To a stirred solution of **22a** (99 mg, 0.177 mmol) in 1:1 dichloromethane/dimethylformamide (2 mL) was added biotin (47 mg, 0.196 mmol), HATU (81 mg, 0.213 mmol), HOBt (33 mg, 0.213 mmol), and DMAP (26 mg, 0.213 mmol). The yellow solution was stirred at 40 °C for 2 days. The volatiles were removed in vacuo, and the residue was purified by silica gel chromatography (1:10 methanol/dichloromethane) to afford **23a** as a colorless oil (70 mg, 50%): $\nu_{max}(CHCl_3)/cm^{-1}$ 1690, 1545; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 6.19 (s, 1H), 6.13−6.02 (m, 1H), 5.44 (s, 1H), 4.54−4.43 (m, 1H), 4.32−4.22 (m, 1H), 3.59−3.07 (m, 15H), 3.01−2.82 (m, 3H), 2.77−2.41 (m, 7H), 2.22−2.11 (m, 2H), 1.80−1.55 (m, 6H), 1.55−1.15 (m, 35H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 173.3 (C), 163.9 (C), 156.2 (C), 155.8 (C), 155.5 (C), 79.5 (C), 79.3 (C), 61.9 (CH), 60.3 (CH), 55.7 (CH), 54.9 (CH_2), 53.6 (CH_2), 52.2 (CH_2), 50.1 (CH_2), 47.9 (CH_2), 47.6 (CH_2), 40.6 (CH_2), 40.1 (CH_2), 39.5 (CH_2), 36.1 (CH_2), 29.6 (CH_2), 28.8 (CH_3), 28.6 (CH_3), 28.3 (CH_3), 28.2 (CH_2), 27.5 (CH), 26.9 (CH_2), 25.8 (CH_2), 23.5 (CH_2). MS (ESI) *m*/*z*: 820[(M + Na)$^+$], 798[(M + H)$^+$]; HRMS (ESI) calcd for $C_{39}H_{71}N_7O_8SNa$ [(M + Na)$^+$] 798.5158, found 798.5156.

10-{3-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoylamino]-propyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (23c). Prepared in an analogous manner to **23a**: **22c** (133 mg, 0.251 mmol), biotin (67 mg, 0.277 mmol), HATU (115 mg, 0.302 mmol), HOBt (46 mg, 0.302 mmol), and DMAP (37 mg, 0.302 mmol) in dichloromethane/dimethylformamide (4 mL) gave **23c** (74 mg, 38%): $\nu_{max}(CHCl_3)/cm^{-1}$ 3304, 2974, 2930, 1686, 1545; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 7.09 (bs, 1H), 6.28 (bs, 1H), 5.62 (bs, 1H), 4.60−4.46 (m, 1H), 4.40−4.26 (m, 1H), 3.79−3.10 (m, 14H), 2.90 (dd, *J* = 13.1, 4.9 Hz, 1H), 2.75 (d, *J* = 12.6 Hz, 1H), 2.81−2.46 (m, 6H), 2.26 (t, *J* = 7.2 Hz, 2H), 1.88−1.57 (m, 6H), 1.88−1.57 (m, 29H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 173.7, 164.0, 156.3, 155.8, 155.3, 79.8, 79.7, 79.6, 61.9, 60.3, 55.7, 55.2, 54.6, 50.7, 50.3, 49.8, 48.5, 40.6, 37.1, 36.0, 28.7, 28.6, 28.4, 28.2, 25.9, 25.1; MS (ESI) *m*/*z*: 778-[(M + Na)$^+$], 756[(M + H)$^+$]; HRMS (ESI) calcd for $C_{36}H_{65}N_7O_8$-SNa [(M + Na)$^+$] 756.4687, found 756.4688.

5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoic Acid [6-(1,4,7,10-Tetraazacyclododec-1-yl)-hexyl]amide-trihydro-trifluoroacetate (24a). Carbamate **23a** (70 mg, 0.09 mmol) was dissolved in dichloromethane (3 mL) and treated with trifluoroacetic acid (0.42 g, 3.69 mmol). The solution was stirred at room temperature overnight and then evaporated to give **24a** (72 mg, 98%): ν_{max}(neat)/cm^{-1} 1673, 1454, 1420; ^{1}H NMR (D_2O, 270 MHz): δ = 4.64−4.54 (m, 1H), 4.42−4.36 (m, 1H), 3.38−3.26 (m, 1H), 3.24−3.06 (m, 16H), 3.02−2.88 (m, 5H), 2.75 (d, *J* = 13.1 Hz, 1H), 2.23 (t, *J* = 6.7 Hz, 2H), 1.72−1.22 (m, 14H); ^{13}C NMR (D_2O, 100 MHz): δ = 175.8, 164.5, 69.0, 61.26, 59.4, 56.3, 54.6, 53.5, 51.9, 48.0, 42.6, 41.7, 40.8, 38.8, 38.2, 34.7, 27.4, 27.0, 26.8, 25.4, 25.1, 25.0, 24.3, 22.2; MS (ESI) *m*/*z*: 498[(M + H)$^+$]; HRMS (ESI) calcd for $C_{24}H_{48}N_7O_2S$ [(M + H)$^+$] 498.3585, found 498.3585.

5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoic Acid [3-(1,4,7,10-Tetraazacyclododec-1-yl)-propyl]-amide-trihydro-trifluoroacetate (24c). Prepared in an identical manner to **24a**: **23c** (74 mg, 0.09 mmol) and trifluoroacetic acid (0.42 g, 3.69 mmol) in dichloromethane (3 mL) gave **24c** (79 mg, 99%) as a colorless oil: ν_{max}(neat)/cm^{-1} 3774, 2928, 2854, 1674, 1454, 1423; ^{1}H NMR (D_2O, 270 MHz): δ = 4.63−4.53 (m, 1H), 4.44−4.34 (m, 1H), 3.36−2.87 (m, 22H), 2.81−2.68 (m, 3H), 2.23 (t, J = 6.9 Hz, 2H), 1.82−1.26 (m, 8H); ^{13}C NMR (D_2O, 100 MHz): δ = 176.9, 165.4, 62.1, 60.3, 55.5, 49.9, 48.9, 48.0, 44.0, 41.9, 39.7, 37.1, 35.4, 27.9, 27.7, 25.1, 23.6; MS (ESI) *m*/*z*: 456 [(M + H)$^+$], 228; HRMS (ESI) calcd for $C_{21}H_{42}N_7O_2S$ [(M + H)$^+$] 456.3115, found 456.3114.

10-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-acetyl]-1,4,7,-10-tetraazacyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (28). To a stirred solution of **16** (145 mg, 0.307 mmol) in a dichloromethane/dimethylformamide mixture (1:1, 2 mL) was added **25** (100 mg, 0.338 mmol), HATU (140 mg, 0.367 mmol), HOBt (56 mg, 0.367 mmol), and DMAP (45 mg, 0.376 mmol). The yellow solution was stirred at 40 °C for 2 days. The solvents were removed in vacuo, and the residue was purified by silica gel chromatography (2:1−1:1 petroleum spirits/ethyl acetate) to afford **28** as a colorless oil (199 mg, 86%): ν_{max}(CH_2Cl_2)/cm^{-1} 3294, 2932, 1694, 1516, 1466; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.75 (d, J = 7.4 Hz, 2H), 7.61 (d, J = 7.4 Hz, 2H), 7.39 (t, J = 7.1 Hz, 2H), 7.30 (t, J = 6.9 Hz, 2H), 5.81 (bs, 1H), 4.38−4.30 (m, 2H), 4.28−4.16 (m, 1H), 4.08−4.00 (m, 2H), 3.62−3.32 (m, 16H), 1.54−1.38 (m, 27H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 168.9, 157.1, 157.0, 156.2, 155.6, 144.0, 141.3, 127.7, 127.1, 125.2, 120.0, 80.6, 80.5, 67.2, 51.5, 49.9, 49.6, 47.2, 42.7, 28.5; MS (ESI) *m*/*z*: 774 [(M + Na)$^+$], 752 [(M + H)$^+$], 652, 552, 452; HRMS (ESI) calcd for $C_{40}H_{57}N_5O_9$-Na [(M + Na)$^+$] 774.4048, found 774.4055.

10-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionyl]-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (29). Prepared in an identical manner to **28**: **16** (750 mg, 1.5 mmol), **26** (544 mg, 1.7 mmol), HOBt (292 mg, 0.1.9 mmol), HATU (725 mg, 1.9 mmol), and DMAP (233 mg, 1.9 mmol) in DMF (2 mL) and DCM (2 mL) afforded **29** (777 mg, 64%) as a colorless oil: ν_{max}(CH_2Cl_2)/cm^{-1} 1693, 1651, 1466, 1414; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.76 (d, J = 7.4 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.7 Hz, 2H), 5.69 (d, J = 7.9 Hz, 1H), 4.63 (apparent q, J = 7.4 Hz, 1H), 4.31 (d, J = 6.9 Hz, 2H), 4.22−4.14 (m 1H), 3.81−3.05 (m, 16H), 1.54−1.31 (m, 30H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 173.3 (C), 157.4 (C), 157.0 (C), 155.6 (C), 155.4 (C), 144.0 (C), 143.9 (C), 141.3 (C), 127.7 (CH), 127.1 (CH), 125.2 (CH), 120.0 (CH), 80.6 (C), 67.0 (CH2), 50.3 (CH_2), 50.0 (CH_2), 49.7 (CH_2), 47.2 (CH), 28.5 (CH_3), 19.8 (CH_3); MS (ESI) *m*/*z*: 788 [(M + Na)$^+$], 766 [(M + H)$^+$], 666, 610, 566, 466; HRMS (ESI) calcd for $C_{41}H_{60}N_5O_9$ [(M + H)$^+$] 766.4386, found 766.4393. [α]^{25} = +21.8 (c = 5.0, CH_2Cl_2).

10-(2-Aminopropionyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (32). Compound **29** (777 mg, 1.02 mmol) was dissolved in a solution of TBAF (641 mg, 2.03 mmol) in acetonitrile (0.05 M) and stirred at room temperature for 90 min, and the reaction was followed by TLC. The reaction was stopped by the addition of dichloromethane (100 mL), the organics were washed with water (2 × 100 mL), and the combined aqueous layers were washed with dichloromethane (2 × 100 mL). The combined organics were dried over $MgSO_4$ and removed in vacuo. The crude material was purified by silica gel chromatography (1:10 methanol/dichloromethane) to give **32** as a colorless oil (408 mg, 74%): ν_{max}(CH_2Cl_2)/cm^{-1} 1689, 1639; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 3.70−3.31 (m, 17H), 2.22 (bs, 2H), 1.48 (s, 9H), 1.46 (s, 18H), 1.20 (d, J = 9.8 Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 176.5 (C), 157.2 (C), 157.0 (C), 155.5 (C), 80.6 (C), 80.5 (C), 80.4 (C), 51.6 (CH_2), 50.4 (CH_2), 49.7 (CH_2), 49.6 (CH_2), 47.1 (CH), 28.5 (CH_3), 21.7 (CH_3); MS (ESI) *m*/*z*: 544 [(M + H)$^+$], 488, 444, 345, 245; HRMS (ESI) calcd for $C_{26}H_{50}N_5O_7$ [(M + H)$^+$] 544.3705, found 544.3705. [α]^{25} = +39.1 (c = 5.0, CH_2Cl_2).

2-Amino-1-(1,4,7,10-tetraazacyclododec-1-yl)-ethanone-tetrahydro-trifluoroacetate (34). Prepared in an identical manner to **35**: **31** (169 mg, 0.32 mmol) and trifluoroacetic acid (0.99 mL, 13.4 mmol) in dichloromethane (10 mL) gave **34** (178 mg, 98%) as a colorless oil: ν_{max}(neat)/cm^{-1} 1670, 1427; ^{1}H NMR (D_2O, 270 MHz): δ = 4.09 (s, 2H), 3.76−3.62 (m, 4H), 3.42−3.12 (m, 12H); ^{13}C NMR (67.5 MHz, D_2O): δ = 169.2, 47.0, 46.4, 45.9, 45.8, 45.0, 44.3, 43.5, 43.4, 40.8; MS (ESI) *m*/*z*: 230 [(M + H)$^+$]; HRMS (ESI) calcd for $C_{10}H_{24}N_5O$ [(M + H)$^+$] 230.1975, found 230.1973.

2-Amino-1-(1,4,7,10-tetraazacyclododec-1-yl)-propan-1-one-tetrahydro-trifluoroacetate (35). Carbamate **32** (408 mg, 0.75 mmol) was dissolved in dichloromethane (33 mL) and treated with trifluoroacetic acid (3.6 g, 31.62 mmol), and the solution was stirred at room temperature overnight and then evaporated to give **35** (404 mg, 98%): ν_{max}(CH_2Cl_2)/cm^{-1} 1674; ^{1}H NMR (D_2O, 270 MHz): δ = 4.47 (q, J = 6.9 Hz, 1H), 4.05−3.05 (m, 16H), 1.52 (d, J = 6.9 Hz, 3H); ^{13}C NMR (D_2O, 67.5 MHz): δ = 172.7(C), 47.9-(CH), 47.4(CH_2), 46.8(CH_2), 46.4(CH_2), 46.2(CH_2), 44.9(CH_2), 44.4(CH_2), 43.6(CH_2), 43.3(CH_2), 15.7(CH_3); MS (ESI) *m*/*z*: 244-[(M + H)$^+$]; HRMS (ESI) calcd for $C_{11}H_{26}N_5O$ [(M + H)$^+$] 244.2132, found 244.2130. [α]^{25} = +10.7 (c = 1.64, MeOH).

2-Amino-1-phenyl-(1,4,7,10-tetraazacyclododec-1-yl)-propan-1-one-tetrahydro-trifluoroacetate (36). Prepared in an identical manner to **35**: **33** (73 mg, 0.118 mmol) and TFA (0.3 mL, 4.96 mmol) in dichloromethane (5 mL) gave **36** (71 mg, 96%); ν_{max}(CH_2-Cl_2)/cm^{-1} 1670; ^{1}H NMR (D_2O, 270 MHz): δ = 7.23−7.10 (m, 3H), 7.08−6.99 (m, 2H), 4.48 (t, J = 7.4 Hz, 1H), 3.86−3.72 (m, 1H), 3.40−2.62 (m, 17H); ^{13}C NMR (D_2O, 67.5 MHz): δ = 170.9, 133.8, 129.3, 128.9, 127.9, 51.7, 46.4, 46.2, 46.0, 45.5, 45.3, 44.1, 43.6, 37.2; MS (ESI) *m*/*z*: 320 [(M + H)$^+$], 242, 120, 102; HRMS (ESI) calcd for $C_{17}H_{30}N_5O$ [(M + H)$^+$] 320.2445, found 320.2444.

10-{2-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoylamino]-acetyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic Acid Tri-*t*-butyl Ester (37). To a stirred solution of **28** (124 mg, 0.235 mmol) in 1:1 dichloromethane/dimethylformamide (4 mL) was added biotin (63 mg, 0.258 mmol), HATU (107 mg, 0.282 mmol), HOBt (43 mg, 0.282 mmol), and DMAP (34 mg, 0.282 mmol). The yellow solution was stirred at 40 °C for 2 days. The solvents were removed in vacuo, and the residue was purified by silica gel chromatography (1:10 methanol/dichloromethane) to give **37** as a colorless oil. (121 mg, 68%): ν_{max}(CH_2-Cl_2)/cm^{-1} 1688, 1470, 1413; ^{1}H NMR ($CDCl_3$, 270 MHz): δ = 6.96 (bs, 1H), 6.29 (bs, 1H), 5.35 (bs, 1H), 4.47 (m, 1H), 4.52−4.43 (m, 1H), 4.37−4.25 (m, 2H), 4.15−3.94 (m, 2H), 3.62−3.25 (m, 16H), 3.18−3.08 (m, 1H), 2.90 (dd, J = 12.6, 4.7 Hz, 1H), 2.73 (d, J = 12.6 Hz, 1H), 2.31−2.18 (m, 2H), 1.82−1.55 (m, 4H), 1.55−1.31 (s, 29H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 173.4, 169.7, 164.2, 157.2, 156.9, 155.7, 80.7, 80.5, 61.8, 60.3, 55.7, 51.4, 50.2, 49.8, 40.1, 40.6, 35.8, 28.5, 28.3, 28.2, 25.7; MS (ESI) *m*/*z*: 778 [(M + Na)$^+$], 628, 572, 528, 428; HRMS (ESI) calcd for $C_{35}H_{61}N_7O_9SNa$ [(M + Na)$^+$] 778.4144, found 778.4144.

5-(2-Oxo-hexahydro-thieno[3,4-*d*-imidazol-4-yl)-pentanoic Acid [2-Oxo-2-(1,4,7,10-tetraazayclododec-1-yl)-ethyl]-amide-trihydro-trifluoroacetate (38). Carbamate **37** (55 mg, 0.07 mmol) was dissolved in dichloromethane (5 mL) and treated with trifluoroacetic acid (0.35 g, 3.07 mmol), and the solution was stirred at room temperature overnight and then evaporated to give **38** (56 mg, 97%): ν_{max}(neat)/cm^{-1} 1682, 1455, 1420; ^{1}H NMR (D_2O, 270 MHz): δ = 4.64−4.55 (m, 1H), 4.46−4.37 (m, 1H), 4.10 (s, 2H), 3.83−3.63 (m, 4H), 3.38−3.14 (m, 12H), 2.98 (dd, J = 12.9, 4.7 Hz, 1H), 2.76 (d, J = 13.1 Hz, 1H), 2.34 (t, J = 7.2 Hz, 2H), 1.78−1.35 (m, 6H); ^{13}C NMR ($CDCl_3$, 270 MHz): δ = 177.9, 173.0, 165.6, 62.5, 60.5, 55.5, 48.9, 47.0, 46.1, 45.5, 45.0, 44.2, 43.6, 42.8, 41.7, 39.8, 35.0, 27.9, 27.8, 25.1; MS (ESI) *m*/*z*: 479 [(M + Na)$^+$], 456 [(M + H)$^+$]. HRMS (ESI) calcd for $C_{20}H_{38}N_7O_3S$ [(M + H)$^+$], 456.2751, found 456.2753.

11-(2-Benzyloxycarbonylamino-acetyl)-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl ester (41). To a solution of **39** (1 mmol, 500 mg) in DCM (30 mL) were added **40** (1 mmol, 209 mg), DCC (1 mmol, 207 mg), and DMAP (1 mmol, 122 mg). The reaction was stirred at room temperature for 3 days, and the white precipitate was filtered. The solution was concentrated in vacuo, and the crude material was purified by flash chromatography on silica gel (EtOAc) to give **41** as a white solid (690 mg, 100%). mp 79−83 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3055, 2985, 1701, 1685, 1654; ^1H NMR ($CDCl_3$, 270 MHz): δ = 7.36−7.27 (m, 5H), 5.79 (bs, 1H), 5.10 (s, 2H), 4.01 (s, 2H), 3.54−3.18 (m, 16H), 1.85−1.64 (m, 4H), 1.44 (s, 18H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 167.8, 156.2, 156.0, 155.5, 136.5, 128.5, 128.1, 128.0, 79.9, 66.8, 50.0, 48.9, 48.4, 47.5, 46.9, 46.5, 46.0, 42.7, 42.5, 28.5, 27.7; HRMS (ES) calcd for $[M + H]^+$, ($C_{35}H_{58}N_5O_9$) 692.4229, found 692.4229.

11-(2-Amino-acetyl)-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (42). Pd/C (10 mol %, 87 mg) was added to **41** (0.81 mmol, 560 mg) dissolved in MeOH (40 mL), and the resulting mixture was stirred under H_2 (1 atm) for 16 h at room temperature. The reaction mixture was filtered through a Celite pad, and the solvent was removed in vacuo to give **42** as a white solid (436 mg, 96%). The product was used without further purification: mp 68−71 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3433, 3055, 2985, 1685; ^1H NMR ($CDCl_3$, 270 MHz): δ = 3.62−3.21 (m, 18H), 1.88−1.62 (m, 4H), 1.44 (s, 27H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 155.8, 155.5, 155.3, 80.3, 80.0, 79.7, 49.4, 48.2, 47.3, 46.9, 46.5, 46.3, 45.9, 44.9, 41.9, 28.3, 27.5; HRMS (ES) calcd for $[M + H]^+$, ($C_{27}H_{52}N_5O_7$) 558.3861, found 558.3855.

11-{2-[5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoylamino]-acetyl}-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (43). Biotin (0.37 mmol, 89 mg), HATU (0.37 mmol, 139 mg), DIPEA (0.38 mmol, 67 μL), and DMAP (3 mg, 10 mol %) were added to a solution of **42** (0.24 mmol, 136 mg) in DMF (10 mL). The solution was stirred for 24 h at room temperature. The solvent was evaporated in vacuo, and the crude material was purified by column chromatography on silica gel with $CHCl_3$/MeOH (95:5 to 90:10) to afford **43** (156 mg, 83%) as a pale yellow solid: mp 83−85 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1693, 1643, 1461, 1265; ^1H NMR ($CDCl_3$, 270 MHz): δ = 7.17 (broad, 1H, NH), 6.66 (broad, 1H, NH), 5.65 (broad, 1H, NH), 4.53−4.43 (m, 1H), 4.34−4.25 (m, 1H), 4.18−3.92 (m, 2H), 3.54−3.20 (m, 16H), 3.17−3.05 (m, 1H), 2.89 (dd, J = 4.2, 12.7 Hz, 1H), 2.73 (d, J = 12.7 Hz, 1H), 2.34−2.20 (m, 2H), 1.87−1.54 (m, 8H), 1.43 (s, 27H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 173.7, 168.8, 164.5, 155.9, 155.6, 80.6, 80.0, 79.9, 61.8, 60.3, 55.8, 50.0−45.0, 40.9, 40.5, 35.8, 28.5, 28.1, 25.7; HRMS (ES) calcd for $[M + H]^+$, ($C_{37}H_{66}N_7O_9S$) 784.4637, found 784.4642.

11-{2-[5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoylamino]-acetyl}-1,4,8,11-tetraaza-cyclotetradecane-trihydro-trifluoroacetate (44). To a solution of **43** (120 mg, 0.15 mmol) in DCM (10 mL), TFA (42 equiv, 6.2 mmol, 460 μL) was added, and the resulting solution was stirred at room temperature overnight. The solvent was evaporated in vacuo to give **43** as a pale yellow solid (112 mg, 91%): mp 45−47 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3340 (broad), 3055, 3985, 1681; ^1H NMR (D_2O, 270 MHz): δ = 4.66−4.56 (m, 1H), 4.48−4.38 (m, 1H), 4.09 (s, 2H), 3.90−3.18 (m, 18H), 3.00 (dd, J = 5.2 and 13.3 Hz, 1H), 2.77 (d, J = 13.3 Hz, 1H), 2.35 (t, J = 7.1 Hz, 2H), 2.30−2.06 (m, 4H), 1.78−1.36 (m, 6H); ^{13}C NMR (D_2O, 67.5 MHz): δ = 177.7, 172.4, 165.5, 163.5 (TFA), 116.5 (TFA), 62.2, 60.5, 55.5, 46.1, 44.6, 44.2, 44.0, 42.7, 42.3, 41.8, 41.4, 39.8, 38.8, 35.2, 27.9, 25.2, 24.0, 19.0; HRMS (ES) calcd for $[M + H]^+$, ($C_{22}H_{42}N_7O_3S$) 484.3064, found 484.3064.

11-(3-Benzyloxycarbonylamino-propyl)-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (45c). Aldehyde **20c** (305 mg, 1.47 mmol) dissolved in THF (5 mL) was added to a solution of **39** (882 mg, 1.76 mmol) in THF (15 mL). Then, $NaBH(OAc)_3$ (934 mg, 4.41 mmol) was added, and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo, and the residue was dissolved in DCM and washed with $NaHCO_3$ (5%) and water. The organic phase was dried over $MgSO_4$, filtered, and concentrated, and the crude material thus obtained was purified by flash chromatography on silica gel (EtOAc) to give **45c** as a white solid (815 mg, 80%): mp 44−47 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3055, 2985, 1710, 1685; ^1H NMR ($CDCl_3$, 270 MHz): δ = 7.36−7.28 (m, 5H), 5.56 (bs, 1H), 5.07 (s, 2H), 3.38−3.14 (m, 14H), 2.57−2.47 (m, 2H), 2.46−2.32 (m, 4H), 1.88−1.54 (m, 6H), 1.44 (s, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 156.4, 155.6, 155.3, 136.8, 128.3, 127.9, 127.8, 79.3, 66.1, 53.4, 52.7, 51.5, 47.7, 47.3, 46.7, 45.9, 39.6, 28.4, 26.6; HRMS (ES) calcd for $[M + H]^+$, ($C_{36}H_{62}N_5O_8$) 692.4593, found 692.4599.

11-(3-Amino-propyl)-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (46c). Pd/C (10 mol %, 121 mg) was added to **45c** (1.13 mmol, 780 mg) dissolved in MeOH (50 mL), and the resulting mixture was stirred under H_2 (1 atm) for 16 h at room temperature. The crude mixture was filtered through a short plug of Celite, and the solvent was removed in vacuo to give **46c** as a white solid (628 mg, 100%), which was used without further purification: mp 47−49 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3055, 2985, 1686; ^1H NMR ($CDCl_3$, 270 MHz): δ = 3.46−3.12 (m, 12H), 2.82−2.30 (m, 10H), 1.94−1.54 (m, 6H), 1.44 (s, 18H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 155.7 (2×C), 155.5 (C), 79.5 (3×C), 53.4 (CH_2), 52.9 (CH_2), 51.4 (CH_2), 48.2 (2×CH_2), 47.4 (m, 4×CH_2), 45.8 (CH_2), 40.2 (CH_2), 28.5 (9×CH_3), 26.6 (m, 2×CH_2); HRMS (ES) calcd for $[M + H]^+$, ($C_{28}H_{56}N_5O_6$) 558.4225, found 558.4228.

11-{3-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoylamino]-propyl}-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (47c). Biotin (374 mg, 1.67 mmol), HATU (634 mg, 1.67 mmol), DIPEA (580 μL, 3.33 mmol), and DMAP (14 mg, 10 mol %) were preactivated in DMF (10 mL) for 10 min at room temperature. Then, **46c** (620 mg, 1.11 mmol) was added, and the yellow solution was stirred for 24 h at room temperature. The solvent was evaporated in vacuo, and the crude material was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 90:10 to 85:15) to give **47c** as a pale yellow solid (450 mg, 52%): mp 109−111 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1685, 1465, 1265; ^1H NMR ($CDCl_3$, 270 MHz): δ = 6.24 (broad, 1H, NH), 5.49 (broad, 1H, NH), 4.53−4.42 (m, 1H), 4.34−4.23 (m, 1H), 3.46−3.04 (m, 15H), 2.96−2.82 (m, 1H), 2.71 (d, J = 12.8 Hz, 1H), 2.58−2.47 (m, 2H), 2.46−2.24 (m, 4H), 2.24−1.98 (m, 4H), 1.92−1.48 (m, 10H), 1.43 (s, 27H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 173.5, 164.1, 155.6, 79.8, 61.9, 60.3, 55.8, 51.8, 48.1−45.5 (m, 7×CH_2), 40.6, 37.5, 36.0, 28.6, 28.4, 28.1, 26.5 (m, 2×CH_2), 25.8; HRMS (ES) calcd for $[M + H]^+$, ($C_{38}H_{70}N_7O_8S$) 784.5001, found 784.5000.

11-{3-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoylamino]-propyl}-1,4,8,11-tetraaza-cyclotetradecane-trihydro-trifluoroacetate (48c). To a solution of **47c** (219 mg, 0.28 mmol) in DCM (10 mL), TFA (42 equiv, 11.7 mmol, 873 μL) was added, and the resulting solution was stirred at room temperature overnight. The solvent was then evaporated in vacuo to give **48c** as a pale yellow solid (229 mg, 100%): mp 41−43 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3294 (broad), 3055, 3985, 1681 (broad); ^1H NMR (D_2O, 270 MHz): δ = 4.61 (dd, J = 4.7 and 7.9 Hz, 1H), 4.41 (dd, J = 4.4 and 7.9 Hz, 1H), 3.66−3.16 (m, 20H), 3.10 (m, 1H), 3.00 (dd, J = 4.7 and 12.8 Hz, 1H), 2.77 (d, J = 12.8 Hz, 1H), 2.28 (t, J = 6.9 Hz, 2H), 2.18−1.98 (m, 4H), 1.97−1.79 (m, 2H), 1.78−1.28 (m, 6H); ^{13}C NMR (D_2O, 100 MHz): δ = 177.6, 165.7, 163.3 (TFA), 116.7 (TFA), 62.4, 60.6, 55.8, 51.9, 49.8, 47.4, 45.2−42.0 (m, 4×CH_2), 40.7 (2×CH_2), 40.0, 36.6, 35.7, 28.3, 28.0, 25.4, 23.9, 20.9, 19.7; HRMS (ES) calcd for $[M + H]^+$, ($C_{23}H_{46}N_7O_2S$) 484.3428, found 484.3430.

11-Carboxymethyl-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (49). To a solution of **39** (500 mg, 1 mmol) in CH_3CN (20 mL) were added Na_2CO_3 (126 mg, 1.1 mmol) and ethyl bromoacetate (134 μL, 1.1 mmol). The mixture

was stirred at reflux for 16 h. The reaction was cooled to room temperature, and the insoluble salts were removed by filtration. The solution was concentrated in vacuo, and the crude material was purified by flash chromatography on silica gel (EtOAc) to give the intermediate ester as a gummy solid (550 mg, 94%): $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2977, 2931, 1732, 1678; ^1H NMR (270 MHz, $CDCl_3$): δ = 4.10 (q, J = 6.9, 14. Hz, 2H), 3.40−3.16 (m, 14H), 2.84−2.78 (m, 2H), 2.66−2.56 (m, 2H), 1.94−1.78 (m, 2H), 1.72−1.58 (m, 2H), 1.43 (s, 27H), 1.23 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 170.9, 155.7, 155.6, 155.5, 79.5 (3×C), 60.2, 55.4, 53.6, 52.8, 51.8, 48.4, 47.4, 47.1, 46.8, 45.3, 28.4 (9×CH_3), 27.0 (2×CH_2), 14.3; HRMS (ES) calcd for $[M + H]^+$, ($C_{29}H_{55}N_4O_8$) 587.4014, found 587.4011. The ester (0.43 mmol, 250 mg) was dissolved in MeOH (5 mL), and a 1 N solution of NaOH (3 mL) added. The solution was stirred for 2 h at room temperature. MeOH was removed in vacuo, and the pH of the remaining aqueous solution was adjusted to 5 by adding a 5% aq solution of citric acid. The aqueous phase was extracted with DCM (3 × 20 mL), and the organic phases were combined, dried over $MgSO_4$, and concentrated in vacuo to give **49** as a white solid (221 mg, 91%): mp 89−91 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3300−2700 (broad), 1685; ^1H NMR (270 MHz, $CDCl_3$): δ = 3.45−3.12 (m, 14H), 2.82−2.48 (m, 4H), 1.94−1.60 (m, 4H), 1.50−1.24 (m, 27H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 172.1, 156.3, 155.3 (2×C), 80.5, 79.9 (2×C), 56.5, 53.9, 52.6, 47.7, 47.5 (2×CH_2), 46.6, 46.0, 28.5 (9×CH_3), 26.4 (2×CH_2); HRMS (ES) calcd for $[M + H]^+$, ($C_{27}H_{51}N_4O_8$) 559.3701, found 559.3705.

11-[(2-Benzyloxycarbonylamino-ethylcarbamoyl)-methyl]-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (51). To a solution of **49** (0.423 mmol, 236 mg) and **50** (164 mg, 0.846 mmol) in DCM (30 mL), DCC (87 mg, 0.423 mmol) and DMAP (52 mg, 0.423 mmol) were added. The mixture was stirred overnight at room temperature, the insoluble salts that had formed were removed, and the resulting solution was washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel (EtOAc/*n*-Hex 7:3 ramping to EtOAc 100%) to give **51** as a white solid (248 mg, 84%): mp 81−83 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1705, 1686; ^1H NMR (270 MHz, $CDCl_3$): δ = 7.37−7.25 (m, 5H), 5.88 (bs, 1H, NH), 5.06 (s, 2H), 3.48−3.18 (m, 18H), 3.05 (s, 2H), 2.66−2.56 (m, 2H), 2.56−2.46 (m, 2H), 1.88−1.60 (m, 4H), 1.44 (s, 18H), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 172.0, 156.9, 155.7 (3×C), 136.7, 128.5, 128.0 (2×CH), 80.3, 80.0 (2×C), 66.6, 59.5, 54.2, 53.0, 48.3, 48.0, 47.5, 47.2, 46.9, 46.8, 41.4, 39.4, 28.5 (9×CH_3), 26.6 (2×CH_2); HRMS (ES) calcd for $[M + H]^+$, ($C_{37}H_{63}N_6O_9$) 735.4651, found 735.4648.

11-[(2-Amino-ethylcarbamoyl)-methyl]-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (52). Pd/C (10 mol %, 34 mg) was added to **51** (0.313 mmol, 230 mg) dissolved in MeOH (15 mL), and the resulting mixture was stirred under H_2 (1 atm) for 16 h at room temperature. The crude mixture was filtered through a short plug of Celite, and the solvent was removed in vacuo to give **52** as a white solid (169 mg, 90%) that could be used without further purification: mp 49−51 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3680, 3433, 1686; ^1H NMR ($CDCl_3$, 270 MHz): δ = 3.66−3.50 (m, 2H), 3.46−3.04 (m, 16H), 2.74−2.48 (m, 4H), 1.90−1.54 (m, 4H), 1.50−1.30 (m, 27H); ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ = 173.1, 155.7 (3×C), 79.8, 79.6 (2×C), 59.6, 54.2, 52.9, 47.7 (2×CH_2), 47.3 (2×CH_2), 46.6, 46.2, 40.5, 37.8, 28.6 (9×CH_3), 26.3 (2×CH_2); HRMS (ES) calcd for $[M + H]^+$, ($C_{29}H_{57}N_6O_7$) 601.4283, found 601.4287.

11-({2-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoylamino]-ethylcarbamoyl}-methyl)-1,4,8,11-tetraaza-cyclotetradecane-1,4,8-tricarboxylic Acid Tri-*t*-butyl Ester (53). To a solution of **52** (0.373 mmol, 223 mg) in DMF (5 mL) were added biotin (136 mg, 0.559 mmol), HATU (212 mg 0.559 mmol), DIPEA (130 μL, 0.746 mmol), and DMAP (5 mg, 10 mol %). The resulting yellow solution was stirred at room temperature for 40 h. The solvent was removed in vacuo, and the crude material was purified by flash chromatography on silica gel ($CHCl_3$/MeOH 9:1 ramping to 8:2) to give **53** as a pale yellow solid (181 mg, 59%): mp 85−87 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1687, 1463, 1419, 1265; ^1H NMR ($CDCl_3$, 270 MHz): δ = 7.28 (bs, 1H, NH), 6.52 (bs, 1H, NH), 5.60 (bs, 1H, NH), 4.53−4.47 (m, 1H), 4.33−4.28 (m, 1H), 3.52−3.20 (m, 16H), 3.18−2.97 (m, 3H), 2.90 (dd, J = 4.9 and 12.8 Hz, 1H), 2.73 (d, J = 12.8 Hz, 1H), 2.68−2.56 (m, 2H), 2.56−2.44 (m, 2H), 2.19 (t, J = 6.6 Hz, 2H), 1.96−1.54 (m, 10H), 1.44 (s, 18H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.0, 171.3, 163.3, 154.6 (3×C), 79.1, 78.9 (2×C), 60.7, 59.2, 58.4, 54.8, 52.1, 51.9, 46.9 (2×CH_2), 46.6 (2×CH_2), 45.8, 45.4, 39.6, 38.8 (2×CH_2), 34.9, 27.8, 27.5 (9×CH_3), 27.4, 27.2, 25.1 (2×CH_2), 24.6; HRMS (ES) calcd for $[M + H]^+$, ($C_{39}H_{71}N_8O_9S$) 827.5059, found 827.5048.

11-({2-[5-(2-Oxo-hexahydro-thieno[3,4-*d*]imidazol-4-yl)-pentanoylamino]-ethylcarbamoyl}-methyl)-1,4,8,11-tetraaza-cyclotetradecane-trihydro-trifluoroacetate (54). To a solution of **53** (0.121 mmol, 100 mg) in DCM (3 mL), TFA (5.08 mmol, 502 μL) was added, and the solution was stirred overnight at room temperature. The solvent was removed in vacuo to give **54** as a pale yellow semisolid (97 mg, 92%): $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3312 (broad), 1682 (broad); ^1H NMR (D_2O, 270 MHz): δ = 4.64 (m, 1H), 4.48−4.40 (m, 1H), 3.46−2.92 (m, 21H), 2.90−2.74 (m, 3H), 2.28 (pseudo-t, J = 6.4 Hz, 2H), 2.10−1.88 (m, 4H), 1.82−1.34 (m, 6H); ^{13}C NMR (D_2O, 100 MHz): δ = 180.1, 176.3, 168.2, 165.8 (TFA), 119.1 (TFA), 65.0, 63.2, 58.3, 57.9, 57.2, 56.2, 50.1, 49.3, 48.4, 47.6, 45.9, 42.6, 42.1, 40.9, 38.4, 36.5, 30.9, 30.6, 28.0, 26.9, 25.5; HRMS (ES) calcd for $[M + H]^+$, ($C_{23}H_{46}N_7O_2S$) 484.3428, found 484.3430.

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Supporting Information Available: Synthesis and characterization data for **6**, **10a**, **10b**, **A**−**C**, **19b**, **22c**, **30**, **31**, **33**, and **50**; ^1H and ^{13}C NMR data for **6**, **10a**,**b**, **11**, **12**, **A**−**C**, **13**, **14**, **18**−**24**, **28**−**32**, **34**−**38**, and **41**−**54**; 2-D NMR data for **23a**; mass spectral data for copper complexes of ligands **35**, **36**, and **38**; and UV−vis data for copper complexes of ligand **35**. This material is available free of charge via the Internet at http://pubs.acs.org.

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