Development and prospects of dedicated tracers for the molecular imaging of bacterial infections

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SUPPORTING INFORMATION

Pretargeting with biotin-(strept)avidin

The biotin-binding protein avidin has been applied as a nuclear infection imaging agent. In 1992 Rusckowski et al. introduced both the imaging of bacteria with ^{111}In-streptavidin as well as a pretargeting approach where first unlabeled streptavidin was injected, followed three hours later by ^{111}In-biotin.[1] Injection of this tracer resulted in a T/NT ratio of 4 (6 h post injection) in an E. coli infected mouse model. In the pretargeting approach the T/NT ratio increased to 13. In a study carried out amongst 15 patients with chronic osteomyelitis a T/NT ratio of 2.7 (10 min to 4 h post injection) was reached with the pretargeting approach.[2] Comparable positive results were reported in other clinical trials with patients suspected of osteomyelitis.[3,4]

However, similar T/NT ratios were reached with injection of ^{111}In-DTPA-biotin alone. ^{111}In-DTPA-biotin scans of over 100 patients with suspected vertebral or post-surgical infections gave comparable results regarding sensitivity and specificity.[5] Also [^{18}F]biotin gave the same results, indicating that biotin alone accumulates in infected sites. A possible explanation is the use of biotin as a growth factor by several bacteria.[6] However, in vitro studies indicated the uptake of biotin by S. aureus to be a passive process.[7] Further studies on the specificity are warranted in well-defined infection and inflammation models.

Transferrin

Transferrin, an iron-transporting protein, has also been applied as infection imaging tracer; this compound functions as an iron source necessary for bacterial growth. Transferrin, lectin and albumin coated QDs (3.5 nm; λem. 530-650 nm) were compared for bacterial staining. The lectin coated QDs showed a clear outer membrane staining, whereas both transferrin and BSA-coated QDs were internalized. Unfortunately the authors did not asses the internalization of these nanoparticles into eukaryotic cell lines to demonstrate their specificity for bacteria.

^{99m}Tc-, ^{111}In- and ^{68}Ga-radiolabeled versions of transferrin have been developed and tested for imaging of infections.[8-10] Kim et al. described that the targeting towards sites of inflammation with radiolabeled transferrin was based on leakage of vessels and receptor targeting on macrophages.[9] Kumar et al, however, used ^{68}Ga-labeled transferrin to target bacteria and described that the bacteria take up transferrin actively to utilize the protein bound iron for their growth. In their animal studies, however, they could not

observe differences between bacterial infections and inflammation.[10] Unfortunately, the few articles published regarding this topic contradict each other making it impossible to draw any conclusions regarding its specificity.

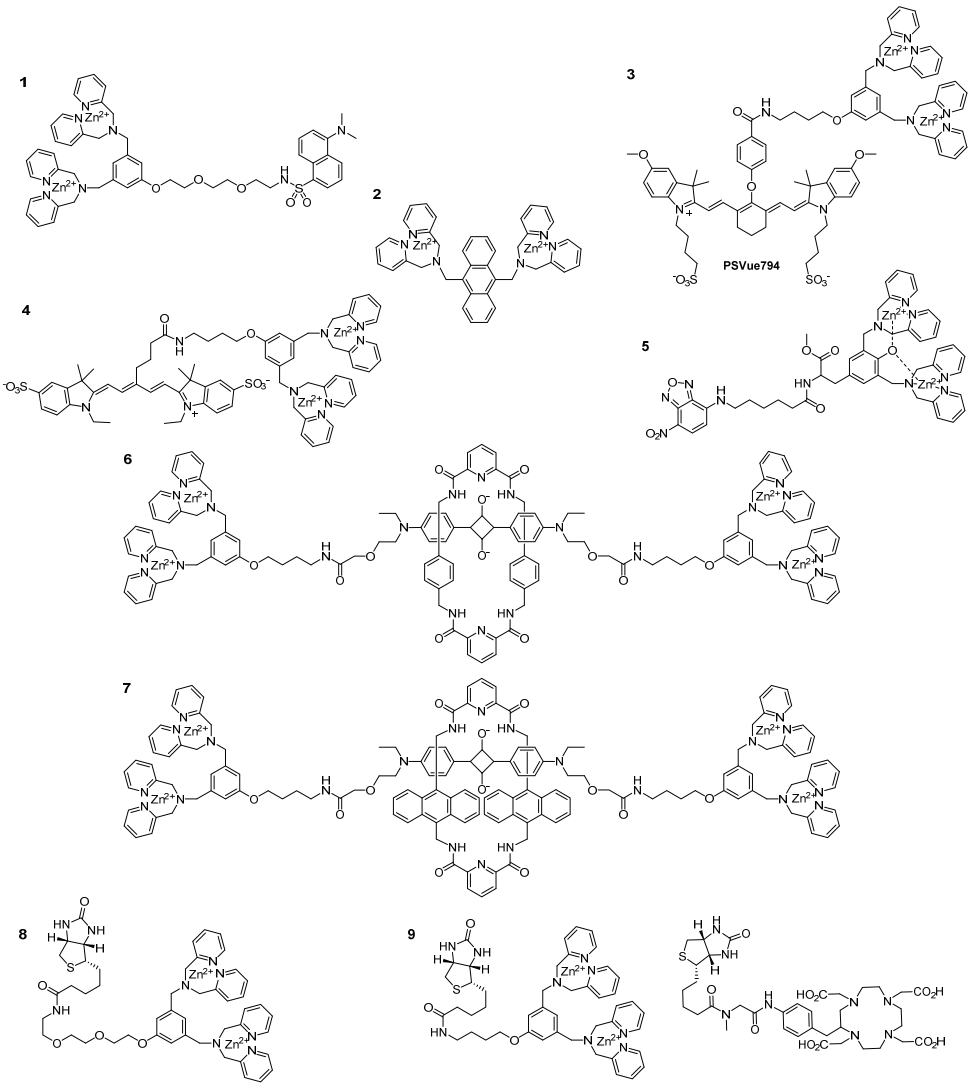
Lysostaphin

Lysostaphin is a metalloendopeptidase produced by S. simulans that targets the pentaglycine linkages within the peptidoglycan layer of gram-positive bacteria and especially of staphylococci. Potapova et al. used both lysostaphin labeled with dyelight488 and a mixed injection of an azide-modified lysostaphin (Lys-N_3) and dibenzocyclooxtyne-Alexa488 .[11] Both approaches resulted in successful labeling of S. aureus and visualization of these bacteria in the bloodstream of mice by intravital fluorescence microscopy.

Immunoglobulins

Antibodies directed against bacteria can be very strong-binding and specific tracers. By combining commercially available streptavidin coated QDs (em. 525 and 705 nm) with biotin-labeled antibodies against E. coli or S. typhimurium, these bacterial strains could be detected separately in a mixture.[12] Also polystyrene nanoparticles embedded with DiI dye (Cy3 derivative) and silica nanoparticles coated with the fluorescent europium complex [Eu(TTA)3(Bpy-Si)] were conjugated to bacteria-specific antibodies. These nanoparticles succeeded in labeling the targeted bacteria in a biofilm.[13,14] Silica nanoparticles incorporating different small fluorescent dyes were conjugated to several antibodies directed against different bacterial strains. These nanoparticles allowed the simultaneous imaging and identification of these strains present in a mixture of species.[15,16]

^{99m}Tc-labeled antibodies directed against S. aureus have been evaluated for their potency in imaging staphylococcal endocarditis in rabbits.[17,18] Although the results were promising, reaching T/NT ratios of 10-120 (24 h post injection), these studies were not continued. Later on, bacterial imaging was performed in various infection models using ^{111}In- and ^{99m}Tc-labeled polyclonal human immunoglobulins.[19] Many clinical studies were carried out with ^{111}In- and ^{99m}Tc-labeled human polyclonal IgG with promising results regarding sensitivity. However, besides a specific interaction of the Fab domains of the antibody with the bacterial cell wall, the interaction of the Fc part of the antibodies to Fc receptors expressed on host immune cells make them possibly non-specific for the imaging of bacterial infections.[20] Even more, circulating antibodies tend to accumulate at sites of inflammation due to increased permeability of the local blood vessels, resulting in aspecific accumulation.[21] This was indicated by the accumulation of the tracers in sterile inflammatory sites.[22] Another drawback of using radiolabeled antibodies are the long blood half-life, so long circulation times of 1-3 days are required to obtain good T/NT ratios.[23]



Scheme 1. Chemical structures of fluorescently and radioactively labeled Zn-DPA derivatives.

Table 1. Binding characteristics of Zn-DPA derivatives

	Ex/em (nm) or isotope	Binding site	In vitro	In vivo	Bacterial strain (gram-status)	Differentiation gram+/gram-	T/NT ratio[a)]	Ref.
1	335/560	Negatively charged bacterial membrane	yes	no	E. coli (-) P. aeruginosa (-) S. aureus (+)	-	-	[24]
2	350/440	"	yes	no	Idem as 1	-	-	[24]
3	794/810	"	yes	yes	E. coli (-) E. faecalis (+) S. lugdunensis (+) S. aureus (+)	-	6 .6 (3 h) 3.7-3.9 (3-21 h)	[25-27]
4	643/658	"	yes	yes	S. aureus (+) E. coli (-) S. typhimurium (-)	-	4.2 (6 h)	[28]
5	470/530	"	yes	no	E. coli (-) S. aureus (+)	-	-	[29]
6	653/675	"	yes	yes	E. coli (-) S. aureus (+) S. enterica (-) K. pneumoniae (-) P. aeruginosa (-) P. vulgaris (-)	-	6.0 (6 h)	[30,31]
7	664/709	"	yes	yes	Idem as 6	-	4.0 (6 h)	[31]
8	<500/565, 655 and 800	"	yes	no	E. coli (-)	+	-	[32]
9	^{111}In	"	no	yes	S. pyogenes (+)	-	2.8 (22h)	[33]

a) T/NT ratio determined relative to opposite limb/thigh.

10 Fitc-TRSSRAGLQFPVGRVHRLLRK(Fitc)-OH
Buforin II

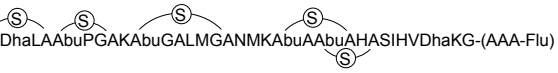
11 Fitc-GIGK(Fitc)FLHSAK(Fitc)K(Fitc)FGK(Fitc)AFVGEIMNS-OH
Magainin 2

12 H_2N-SWLSKTAKKLENSAKKRISEGIAIAIQGGPRC(Cy5)-OH
Cecropin P1

13 H_2N-RGLRRLGRKIAHGVKKYGPTVLRIIRIAGC(Cy5)-OH
SMAP-29

14 H_2N-GVLSNVIGYLKKLGTGALNAVLKQC(Cy5)-OH
PGQ

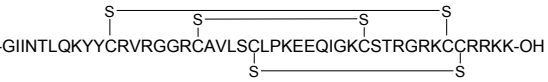
15 H_2N-RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFPC(Bodipy/Alexa680)-OH
Bac7 (1-35)

16 

Nisin

17 

HNP-1

18 

HBD-3

19 ^{99m}Tc H_2N-GRRRRSVQWCA-OH
hLF 1-11

20 **a** ^{99m}Tc H_2N-DSHAKRHHGYKRKFHEKHHSHRGY-OH
Histatin

b ^{99m}Tc H_2N-KRKFHEKHHSHRGY-OH
Dh5

c ^{99m}Tc H_2N-KRLFKKLLFSLRKY-OH
Dhvar4

d ^{99m}Tc H_2N-LLLFLLKKRKKRKY-OH
Dhvar5

e ^{99m}Tc $\alpha,\varepsilon(Dh5)_2K$-$NH_2$
Dh5 dimer

f ^{99m}Tc $\alpha,\varepsilon(Dhvar4)_2K$-$NH_2$
Dhvar4 dimer

g ^{99m}Tc $\alpha,\varepsilon(Dhvar5)_2K$-$NH_2$
Dhvar5 dimer

21 **a** ^{64}Cu-DOTA-(*N*Lys*N*spe*N*spe)$_4$-NH_2

b ^{64}Cu-DOTA-(*N*Lys*N*spe*N*spe)$_3$*N*Lys*N*spe-NH_2

c ^{64}Cu-DOTA-*N*Lys*N*spe*N*pm(*N*Lys*N*pm*N*pm)$_3$-NH_2

Scheme 2. Structures of antimicrobial peptides developed or used for imaging bacterial infections.

22 **a** H_2N-TGRAKRRMQYNRR-OH

b ^{99m}Tc H_2N-TGRAKRRMQYNRR-OH

c ^{99m}Tc-MAG_3-TGRAK(^{99m}Tc-MAG_3)RRMQYNRR-OH

d ^{99m}Tc-Hynic-TGRAKRRMQYNRR-OH

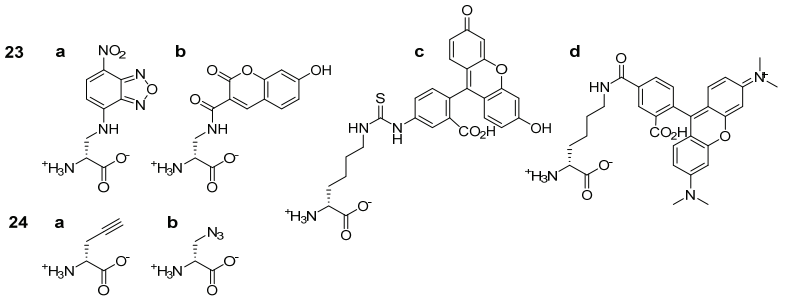
e ^{99m}Tc-N_2S_2-TGRAKRRMQYNRR-OH

f H_2N-TGRAKRRMQY([^{123}I])NRR-OH

g [^{18}F]Bz-TGRAK([^{18}F]Bz)RRMQYNRR-OH

h ^{68}Ga-NOTA-GRAKRRMQYNRR-OH

Scheme 3. Radioactively labeled derivatives of UBI_{29-41}.

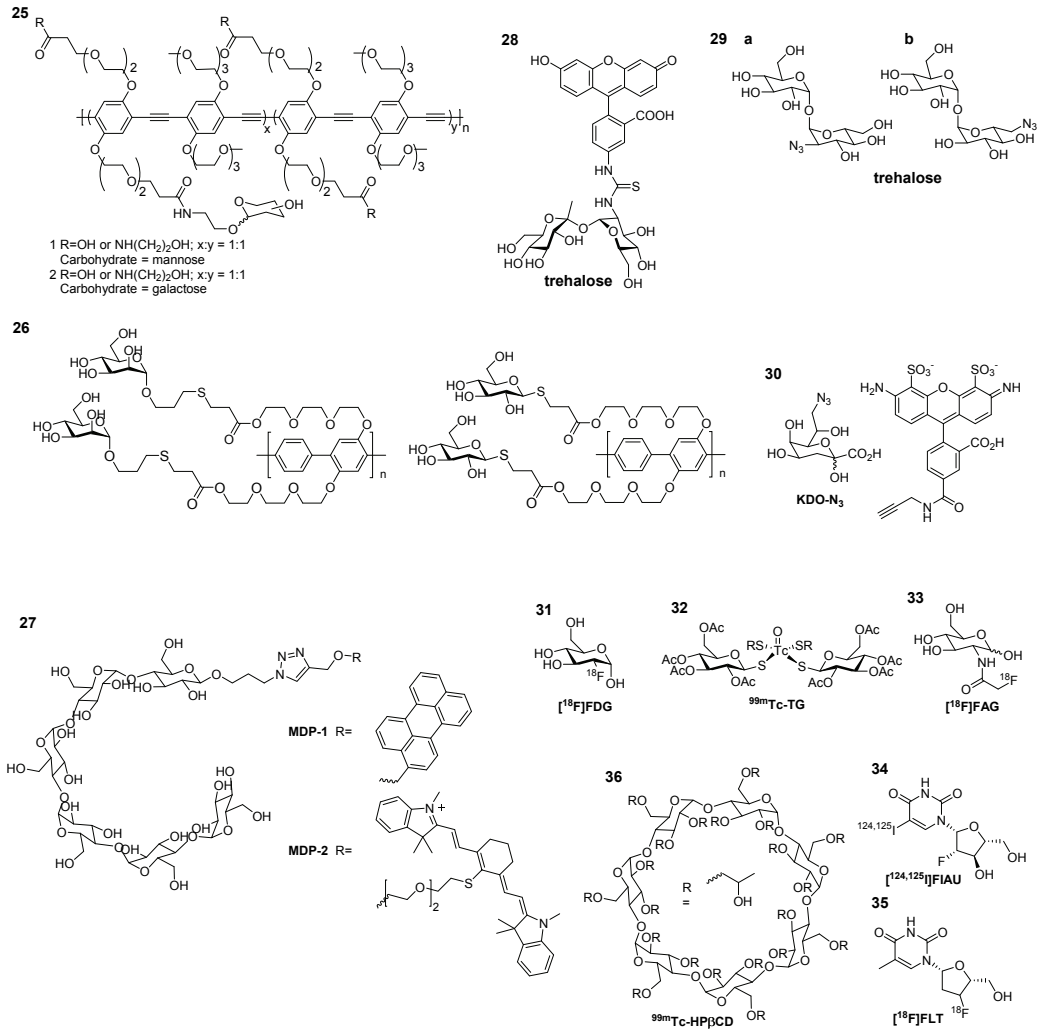


Scheme 4. Fluorescently labeled and clickable D-amino acid derivatives

Table 2. Binding characteristics of antimicrobial peptides

	Ex/em (nm) or isotope	Binding site	In vitro	In vivo	Bacterial strain (gram-status)	Differentiation gram+/gram-	T/NT ratio[b)]	Ref
10	494/521	Bacterial membrane	yes	no	E. coli (-)	-	-	34
11	"	"	yes	no	E. coli (-)	-	-	34
12	650/670	Phospholipids	yes	no	E. coli (-)	+	-	35
13	"	Bacterial membrane	yes	no	E. coli (-)	-	-	35
14	"	-	yes	no	E. coli (-)	-	-	35
15	488/525	SbmA/BacA	yes	no	E. coli (-)	+	-	36
	679/702		yes	yes[a]	S. enterica (-)			37
16	494/521	Lipid II	yes	no	B. subtilis (+) B. megaterium (+)	+	-	38
17	^{99m}Tc	Bacterial membrane	yes	yes	S. aureus (+) K. pneumoniae (-)	-	4.3 (15min) 2.0 (15 min)	39
18	"	"			S. aureus (+) E. coli (-)	-	3 (3-5 h)	40
19	"	"	yes	yes	S. aureus (+) K. pneumoniae (-)	-	2.6 (1 h) 3.5-4 (1 h)	41,42
20	"	"	yes	yes	S. aureus (+)	-	2-4.4 (1 h)	43
21	^{64}Cu	"	yes	yes[a]	E. coli (-)	-	-	44
22	^{99m}Tc, ^{18}F, ^{123}I, ^{68}Ga	"	yes	yes	S. aureus (+) K. pneumoniae (-)	-	2-5 (1-2 h)	45-60
23	404/450 448/538 494/521 550/580	Peptidoglycan synthesis	yes	no	Multiple	-	-	61
24	495/520 550/570 546/565	"	yes	no	E. coli (-) L. monocytogenes (+) C. glutamicum (+) M. tuberculosis (+/-)	-	-	61,62

a) only in healthy uninfected mice; b) T/NT ratio determined relative to opposite limb/thigh.

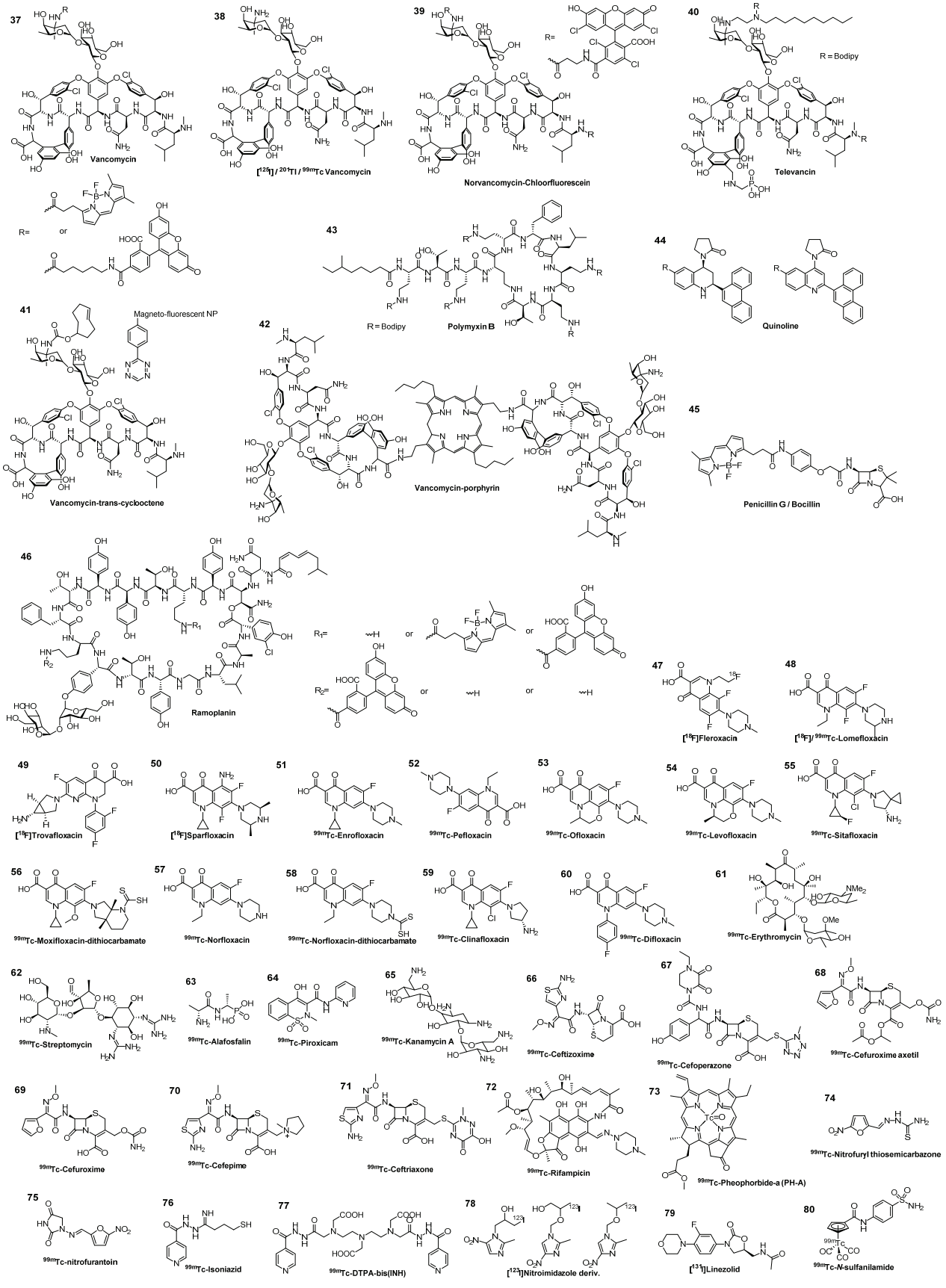


Scheme 5. Structures of carbohydrates developed for imaging infections.

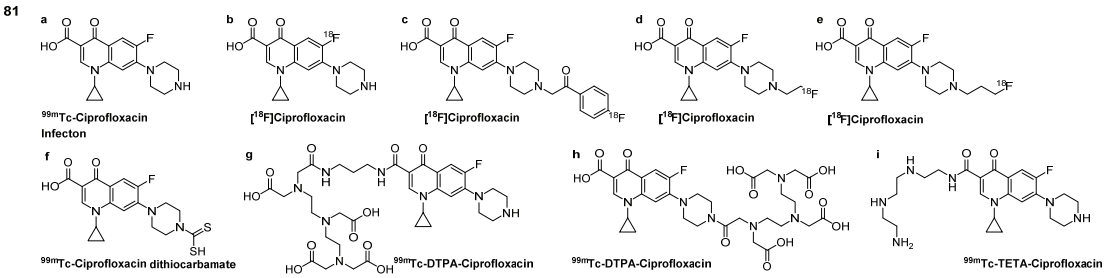
Table 3. Binding characteristics of carbohydrates

	Ex/em (nm) or isotope	Binding site	In vitro	In vivo	Bacterial strain (gram-status)	Differentiation gram+/gram-	T/NT ratio[a)]	Ref
25	364/510	FimH	yes	no	E. coli (-)	+	-	63
26	339/440	FimH	yes	no	E. coli (-)	+	-	64
27	410/450 770/785	Maltodextrin transporter	yes	yes	E. coli (-) P. aeruginosa (-) B. subtilis (+) S. aureus (+)	-	26 (6 h)	65
28	494/521	Ag85 A-C	yes	no	M. tuberculosis (+/-)	+	-	66
29	495/519	Ag85 A-C	yes	no	M. smegmatis (+/-)	+	-	67
30	495/519	CMP-KDO synthetase	yes	no	E. coli (-) S. typhimurium (-) L. pneumophila (-)	+	-	68
31	^{18}F	Glucose metabolism	yes	yes	-	-	-	69,70
32	^{99m}Tc	Glucose metabolism	yes	yes	S. aureus (+)	-	3.2-4.2 (2 h)	71
33	^{18}F	Peptidoglycan synthesis	yes	no	E. coli (-)	-	2.8 (1 h)	72
34	^{125}I	Thymidine kinase	yes	yes	E. coli (-) E. faecalis (+) S. aureus (+) S. epidermidis (+) S. pneumoniae (+) C. novyi (+)	-	> 14 (16 h)	73-75
35	^{18}F	Thymidine kinase	yes	yes	S. typhimurium (-)	-	12.3 (2 h)	76
36	^{99m}Tc	Maltose binding protein	no	yes	E. coli (-)	-	-	77

a) T/NT ratio determined relative to opposite limb/thigh.



Scheme 6. Chemical structure of antibiotics developed or used for bacterial infection imaging.

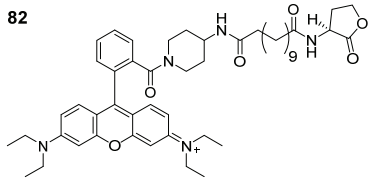


Scheme 7. Radioactively labeled ciprofloxacin derivatives.

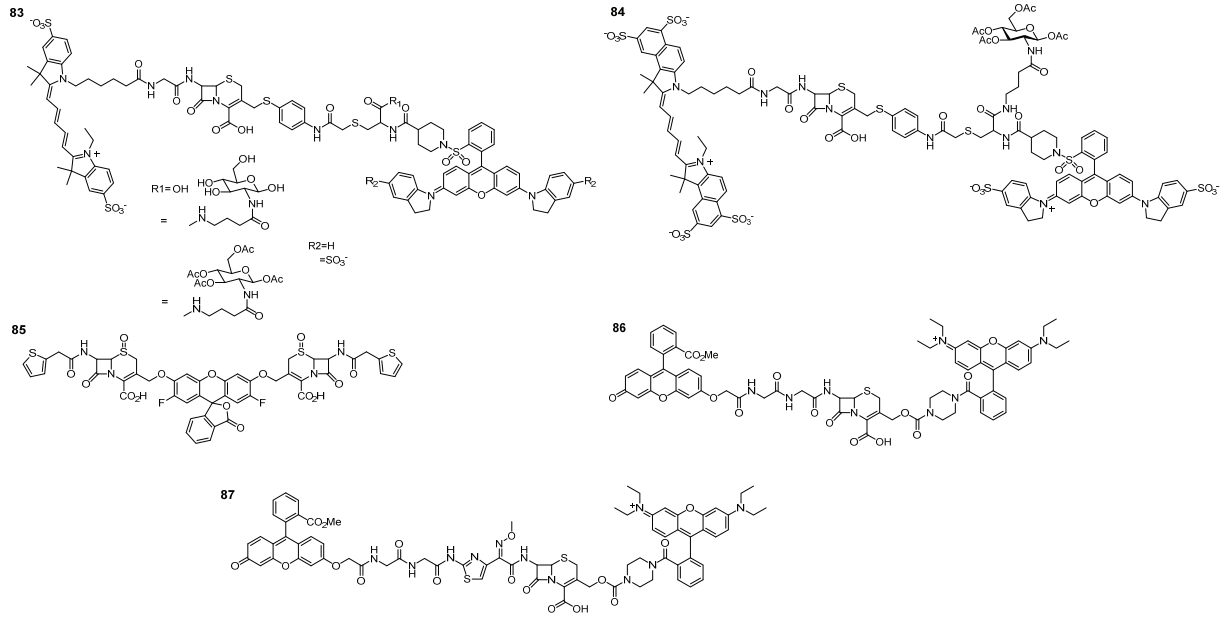
Table 4. Binding characteristics of a selection of antibiotic-based infection tracers

	Ex/em (nm) or isotope	Target	In vitro	In vivo	Bacterial strain (gram-status)	Differentiation gram+/gram-	T/NT ratio[a)]	Ref
38	^{99m}Tc	Lipid II	yes	yes	S. aureus (+)	-	5 (1h)	78
70	"	PBPs	yes	yes	E. Coli (-)	-	8.4 (3h)	79
72	"	RNA polymerase	no	yes	S. aureus (+)	-	7.3 (90 min)	80
73	"	-	yes	yes	S. aureus (+)	-	5.6 (1h)	81
76	"	InhA	yes	yes	M. tuberculosis (+/-)	+	3.5 (24h)	82
77	"	InhA	yes	yes	M. tuberculosis (+/-)	+	4.5 (4 h)	83
79	^{131}I	protein synthesis complex	no	yes	S. aureus (+)	+	77.5 (30min)	84

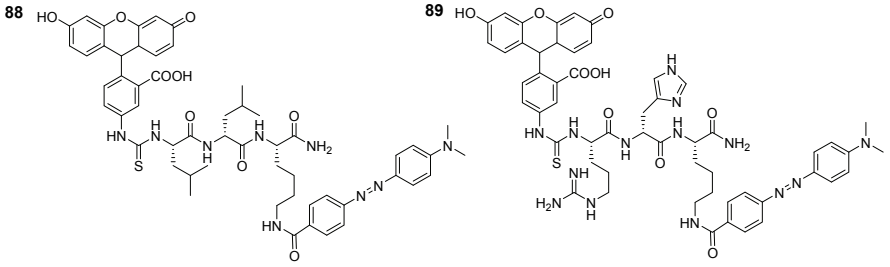
a) T/NT ratio determined relative to opposite limb/thigh.



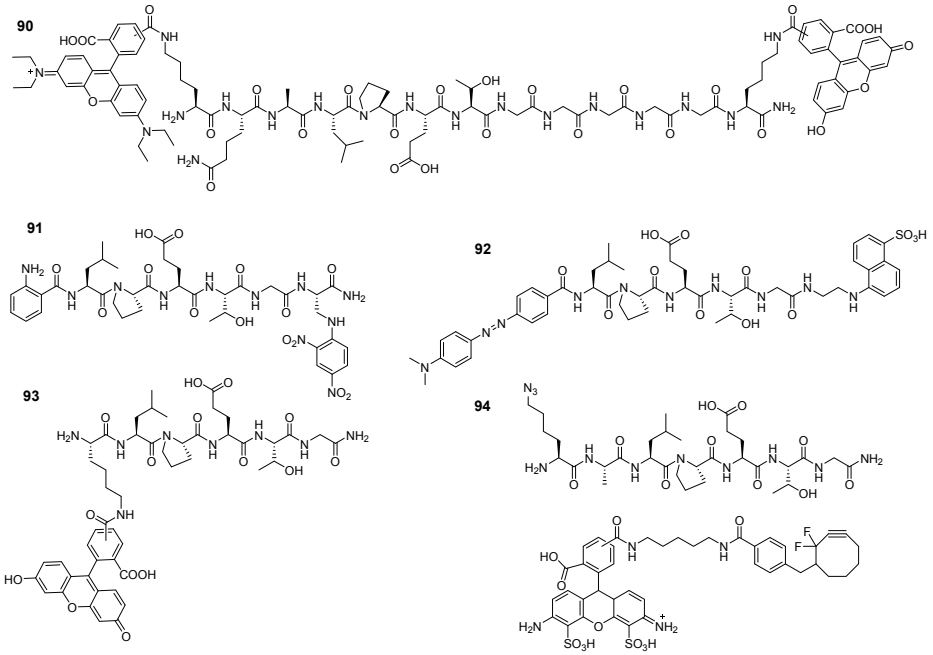
Scheme 8. Fluorescently labeled AHL.



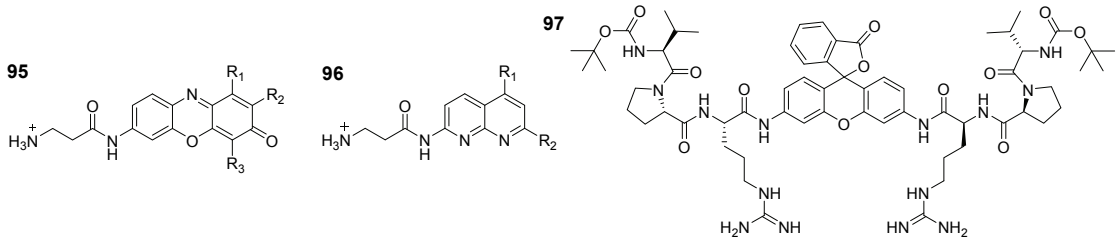
Scheme 9. Structures of lactamase activatable fluorescent tracers developed or used for bacterial imaging



Scheme 10. Structures of protease activatable fluorescent tracers used for bacterial imaging



Scheme 11. Structures of sortase activatable fluorescent tracers developed or used for bacterial imaging



Scheme 12. Structures of β-alanylaminopeptidase activatable tracers developed for bacterial imaging

Table 5. Binding characteristics of QS and enzyme activatable infection probes

	Ex./Em. (nm) or isotope	Binding site	In vitro	In vivo	Bacterial strain (gram-status)	Differentiation gram+/gram-	T/NT ratio	Ref.
82	560/600	CepR	yes	No	E. coli (-) B. cenocepacia (-)	+	-	[85]
83	650/670	β-lactamase	yes	no	-	-	-	[86]
84	670/690	"	yes	yes	M. tuberculosis (+/-) P. aeruginosa S. aureus	-	-	[87]
85	496/524	"	no	no	-	-	-	[88]
86/87	440/520-590	"	yes	no	E. cloacae (-) K. pneumoniae (-) E. coli (-) E. group 137 (-)	-	-	[89]
88	495/520	D-amino acid protease	yes	no	Multiple	-	-	[90]
89	495/520	"	yes	no	Multiple	-	-	[91]
90	450/530 520/585	Sortase	no	no	-	+	-	[92]
91	495/520	"	yes	no	S. aureus(+)	+	-	[93]
92	317/420	"	no	no	-	+	-	[94,95]
93	350/495	"	no	no	-	+	-	[96]
94	499/519	"	yes	no	S. aureus (+)	+	-	[93]
96	374/406	β-alanylaminopeptidase	yes	no	multiple		-	[97]
97	492/523	Staphylocoagulase/prothrombin	Yes	No	S. aureus (+)	-	-	[98]

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