



LIFE FROM INSIDE

**Giornata di Studio sullo Sviluppo e Sicurezza dei Processi Chimici**  
**Milano (NH Hotel Rho-Fiera) - 24 Maggio 2011**



**Bile acid at work:  
development of Sodium Gadocoletate as a new  
intravascular MRI contrast agent**

**Massimo Visigalli – Bracco Imaging SpA**  
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# Bracco Imaging SpA



**1927** In Milan, Elio Bracco founds Italmerck SpA, the Italian manufacturing company of E. Merck that becomes Bracco Industria Chimica in the late '40s



**'60s-'70s** Bracco intensifies research and development in imaging diagnostics



**'80s-'90s** Bracco starts the internationalization process and founds two new R&D units in Geneva (Switzerland) and Princeton (USA)



**2001** All imaging activities (R&D, production, marketing) are grouped under a dedicated company Bracco Imaging

**2007** Bracco Imaging opens a new R&D Center in Colleretto Giacosa (TO) c/o Bioindustry Park S. Fumero

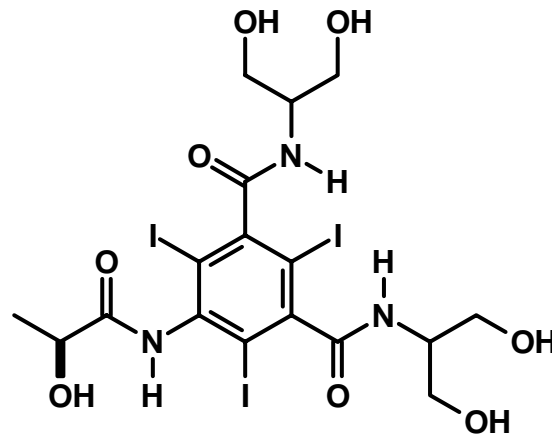


# Bracco Imaging SpA

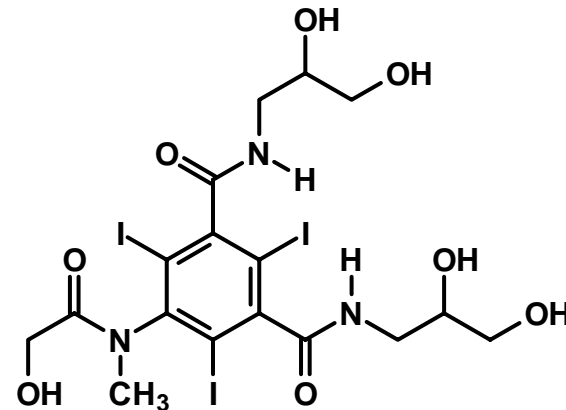


Worldwide leader in contrast agents for Imaging protocols

☞ X-Ray



**Iopamidol**  
**(Iopamiro®)**



**Iomeprol**  
**(Iomeron®)**

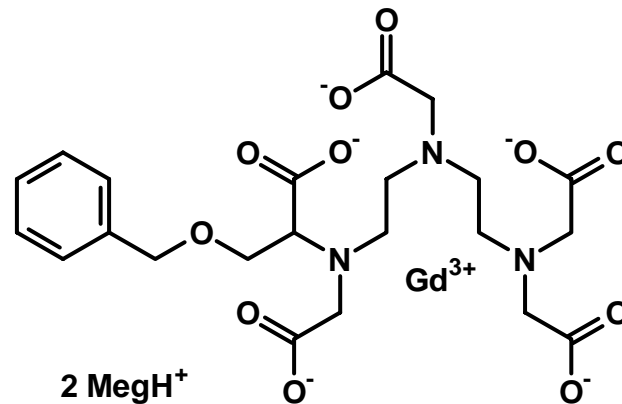
# Bracco Imaging SpA



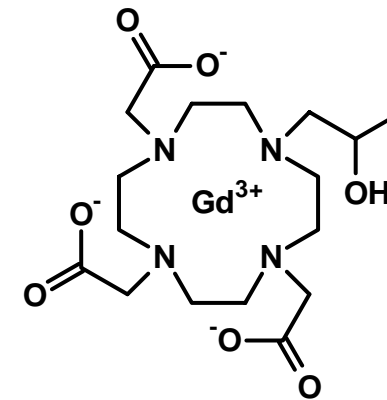
Worldwide leader in contrast agents for Imaging protocols

☞ X-Ray – Iopamidol, Iomeprol

☞ MRI



**Gadobenate dimeglumine**  
**(MultiHance®)**



**Gadoteridol**  
**(ProHance®)**

# Bracco Imaging SpA

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**Worldwide leader in contrast agents for Imaging protocols**

☞ **X-Ray Iopamidol, Iomeprol**

☞ **MRI Gadobenate dimeglumine, Gadoteridol**

☞ **US Sulphur hexafluoride (Sonovue®)**

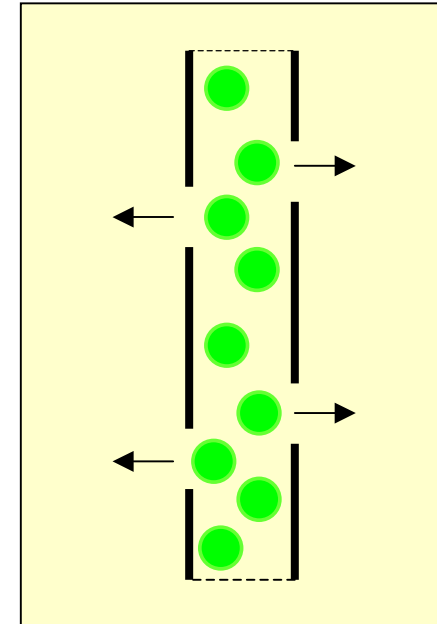
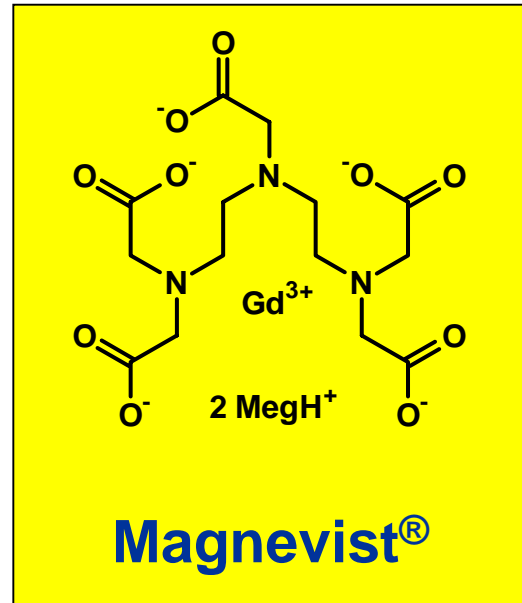
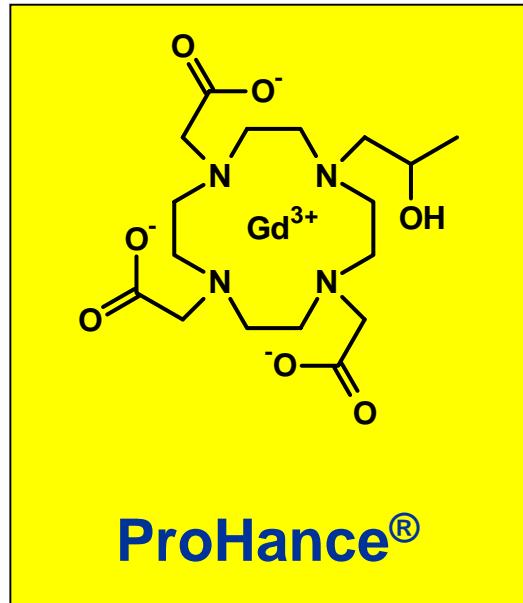
# Intravascular MRI contrast agents

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1. “Why”? and “how”?
2. Bile acids at work
3. Choice and development of the clinical candidate

# Extracellular MRI contrast agents



After *i.v.* injection:

- They diffuse into the interstitial space
- They diffuse and are quickly eliminated through the kidneys



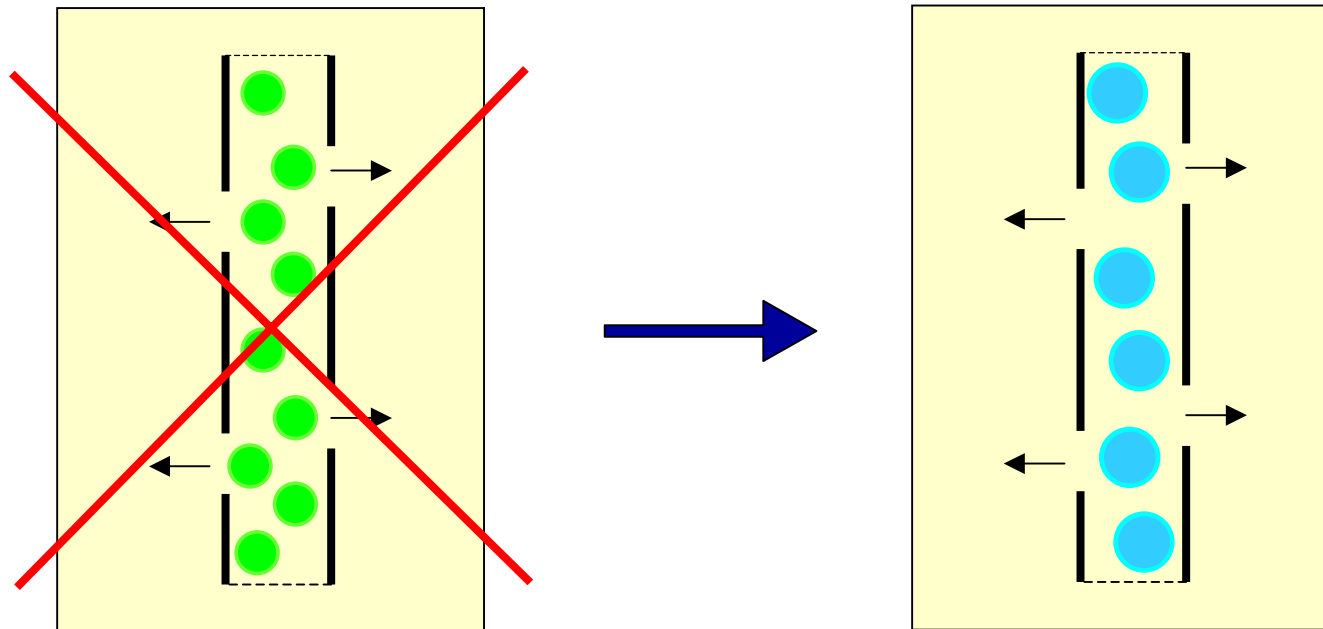
# MRI angiography – Blood Pool Agents (BPA)



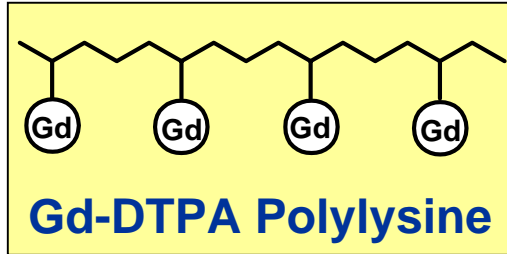
## Coronary angiography:

### ➤ difficulties:

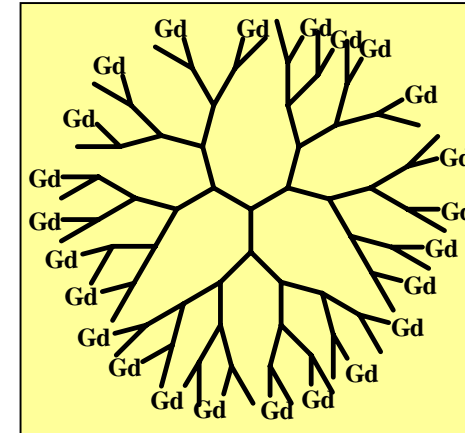
- nature of the blood vessels
- cardiac and respiratory motion effects



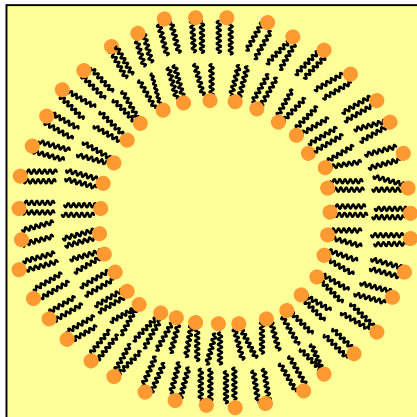
# BPA: approaches



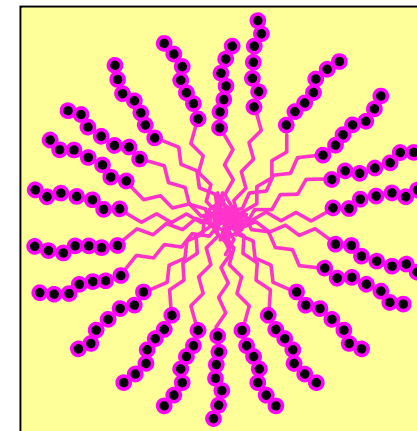
**Polymers**



**Dendrimers**

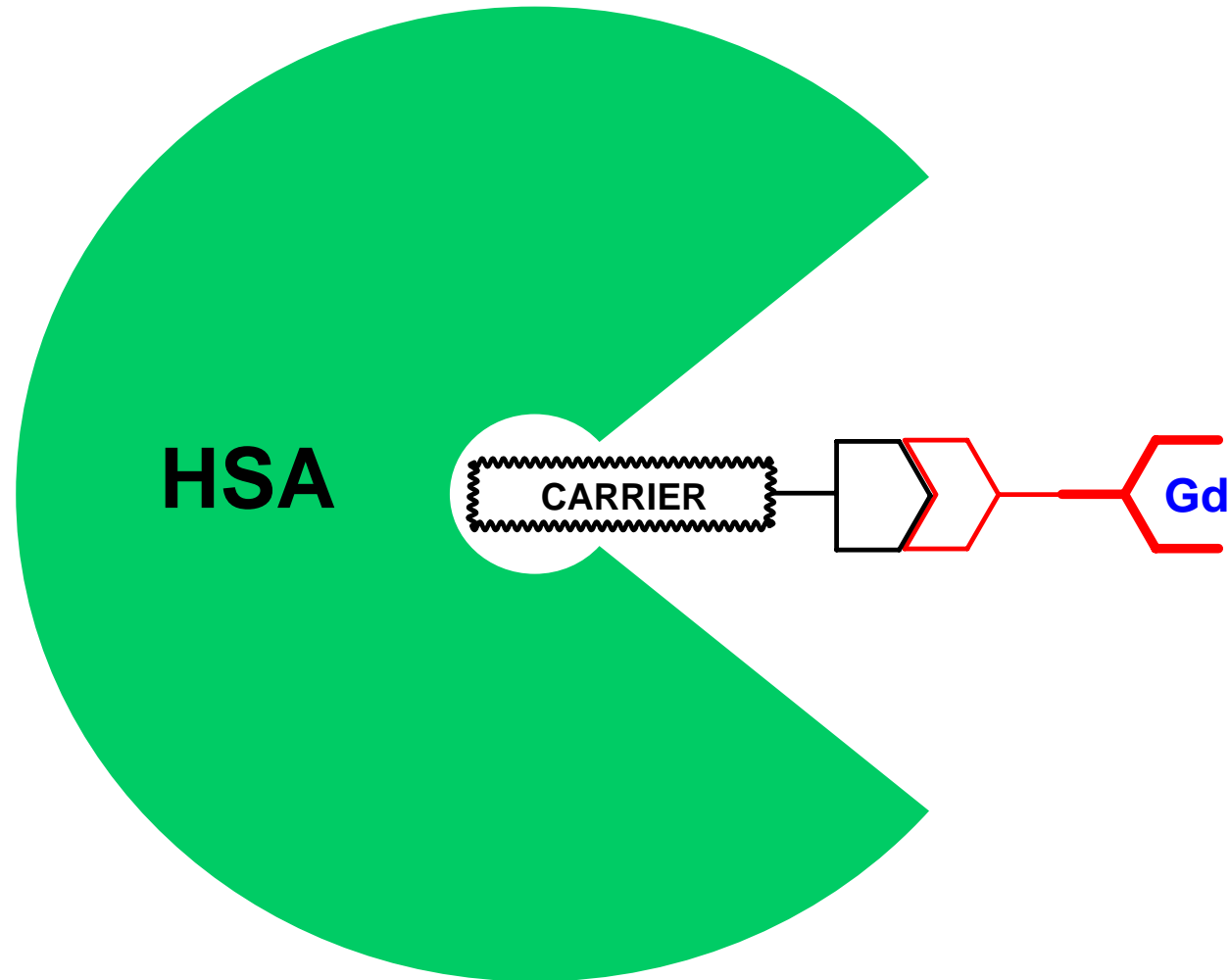


**Liposomes**

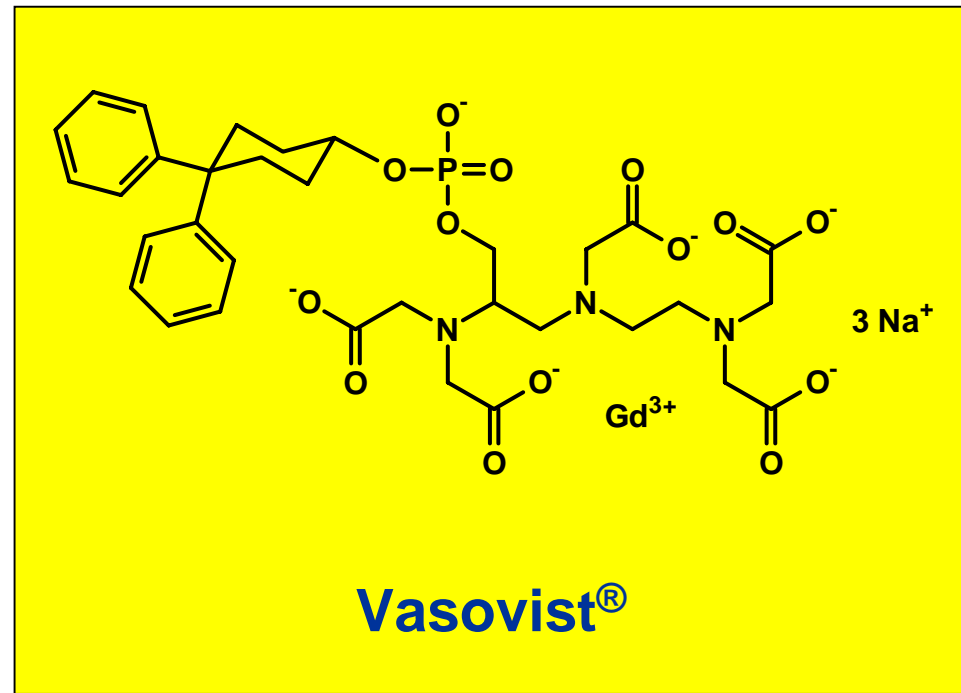


**Micelles**

# BPA: Protein Binders



# BPA: Protein Binders



# Intravascular MRI contrast agents

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1. “Why”? and “how”?

2. Bile acids at work

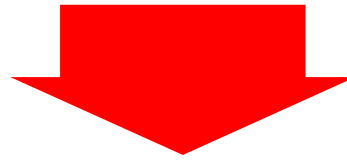
3. Choice and development of the clinical candidate

# Learning from the past

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## Hepatocyte-directed contrast agents



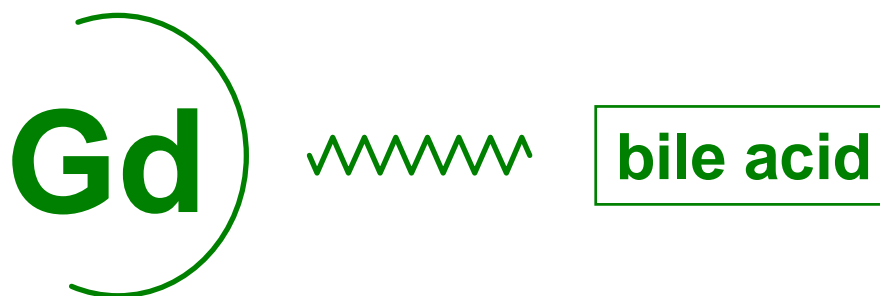
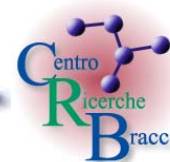
Gd complexes which are transported into hepatocytes by the

**Na<sup>+</sup>/taurocholate transporter**

in the basolateral membrane

# Conjugates of Gd-complexes to Bile Acids

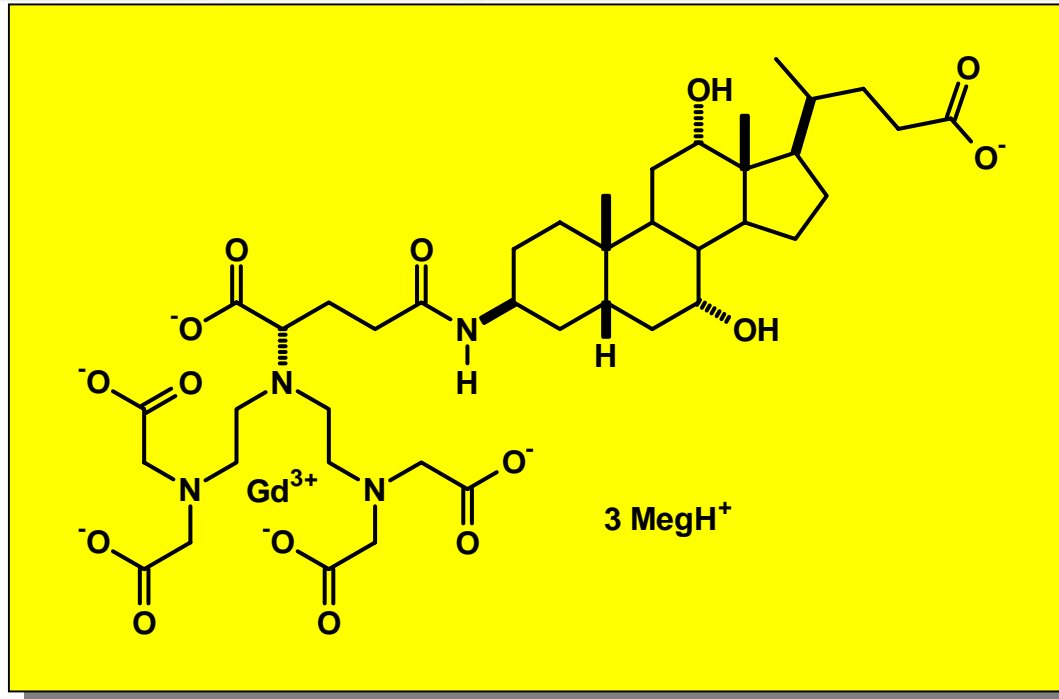
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- Nature of the bile acid
- Site of conjugation on the bile acid skeleton
- Nature of the Gd-complex



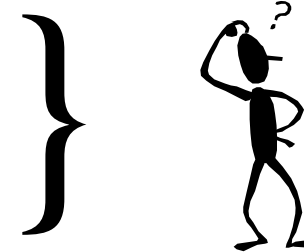
# BPA: Lead compound?



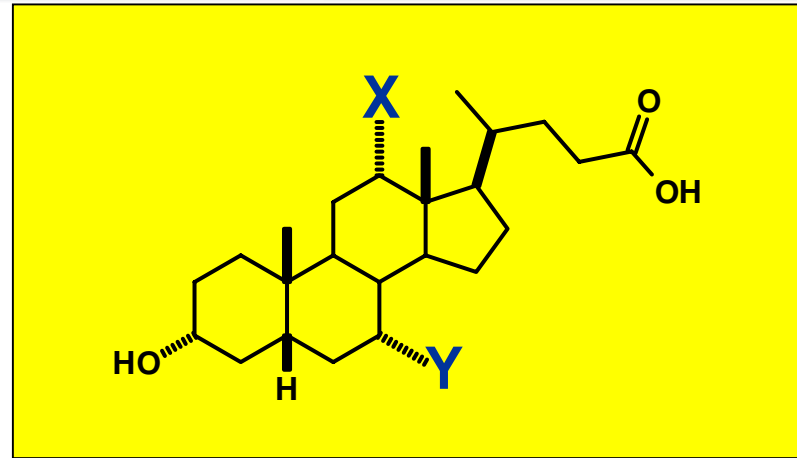
Binding to HSA: 72 % ( $K_a$   $7.4 \cdot 10^3$ , M<sup>-1</sup>)

[ $r_1$  in human serum:  $19.8$  mM<sup>-1</sup>s<sup>-1</sup>]

LD<sub>50</sub>:  $7.6$  mmol kg<sup>-1</sup>

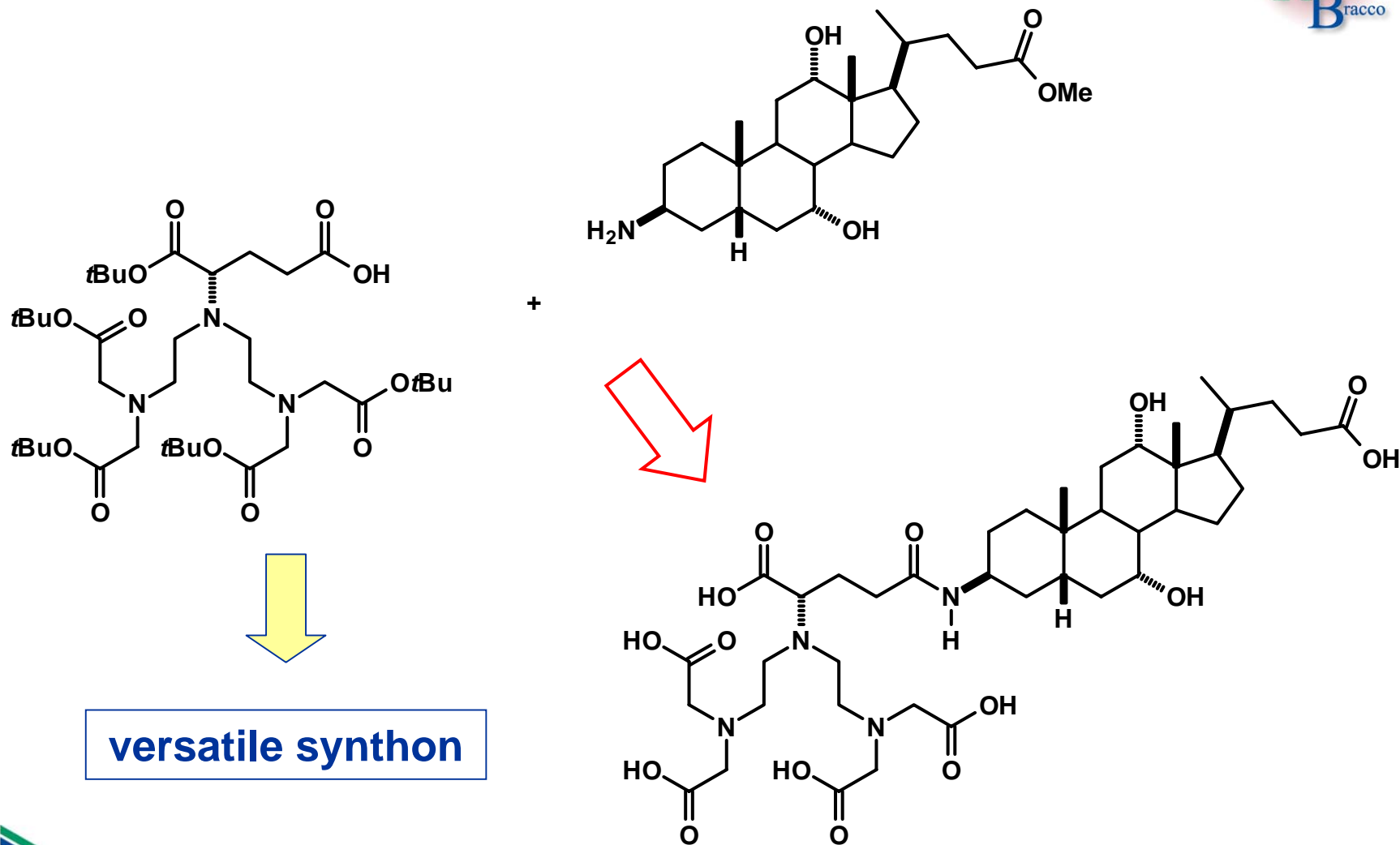


# Bile acids as HSA binders



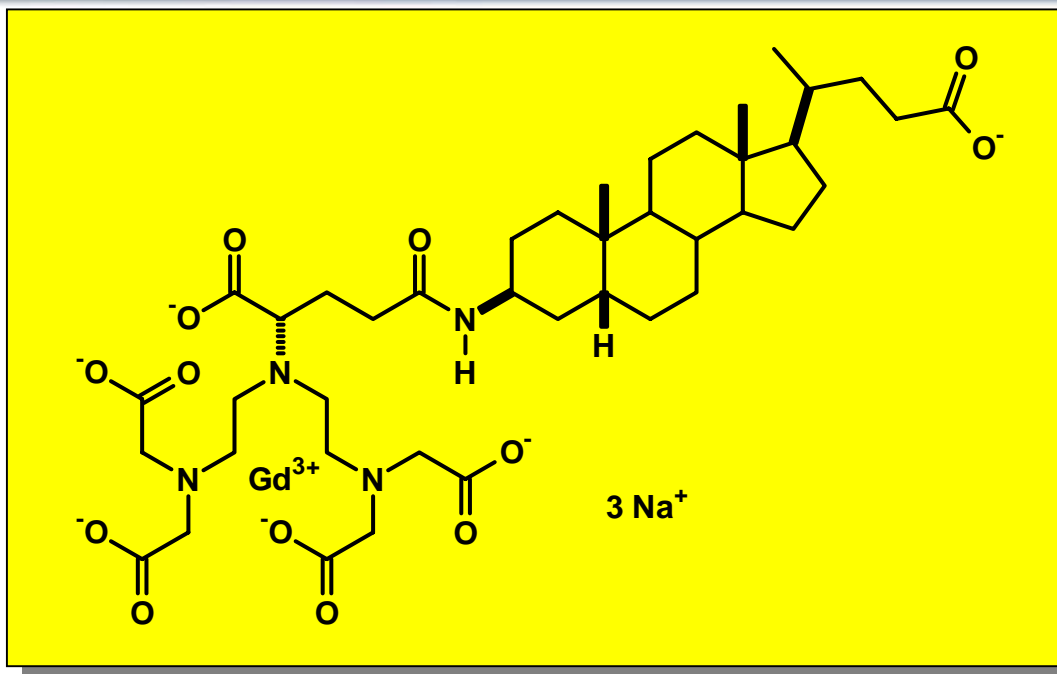
	X	Y	HSA Bind. ( $K_a \cdot 10^4, M^{-1}$ )
Cholic a.	OH	OH	0.33
Deoxycholic a.	OH	H	4.0
Chenodeoxycholic a.	H	OH	5.5
Lithocholic a.	H	H	20

# A versatile approach



versatile synthon

# Strengthen HSA binding...



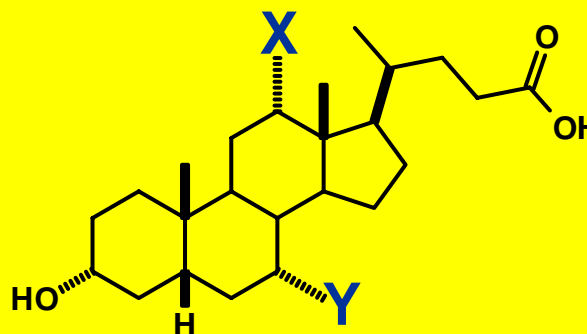
Binding to HSA: 98 % ( $K_a > 10^{10}, M^{-1}$ )

[ $r_1$  in human serum:  $29 mM^{-1}s^{-1}$ ]

**$LD_{50}$ :  $0.66 mmol kg^{-1}$**   
**Elimination: unsatisfactory!**



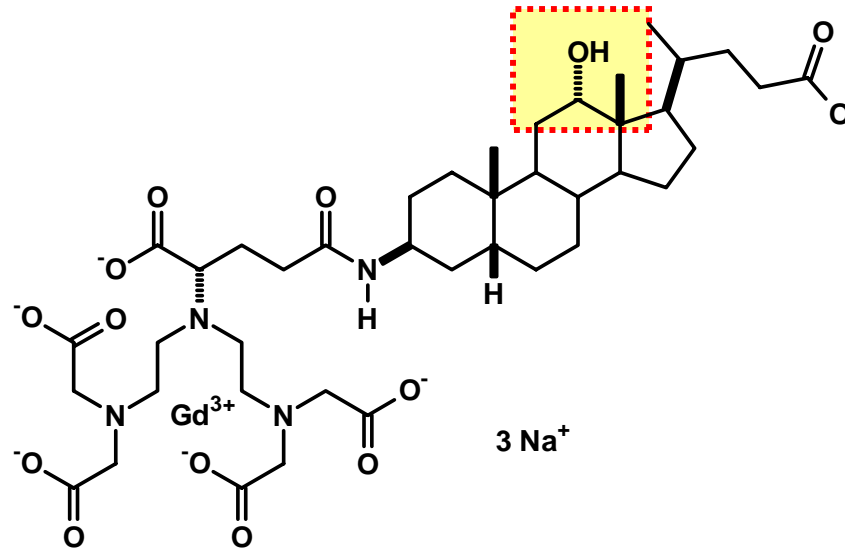
# Bile acids as HSA binders



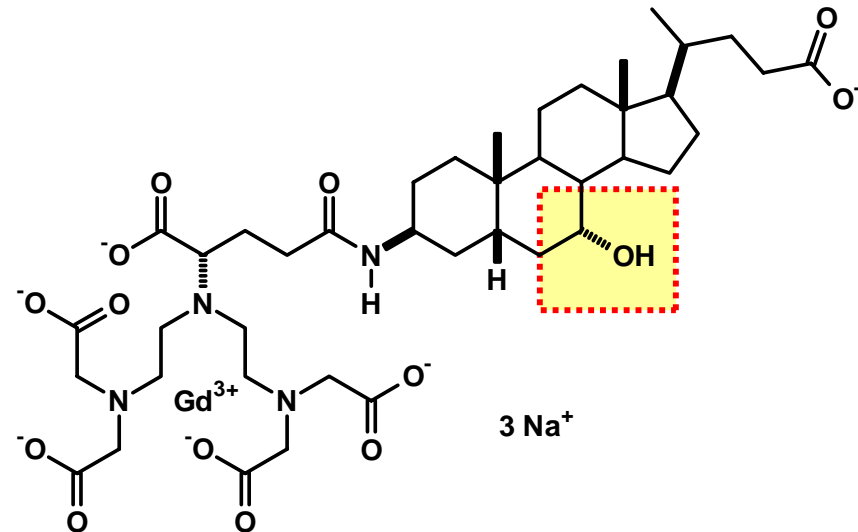
	X	Y	HSA Bind. ( $K_a \cdot 10^4, M^{-1}$ )
Cholic a.	OH	OH	0.33
Deoxycholic a.	OH	H	4.0
Chenodeoxycholic a.	H	OH	5.5
Lithocholic a.	H	H	20

# Looking for the right binding

Deoxycholic  
derivative



Chenodeoxycholic  
derivative



# Intravascular MRI contrast agents

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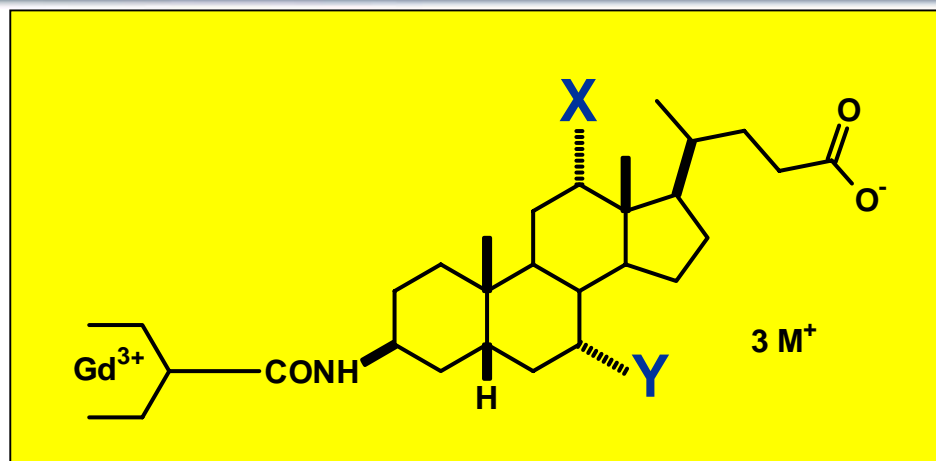


1. “Why”? and “how”?

2. Bile acids

3. Choice and development of the clinical candidate

# Preliminary screening



Derivative	X	Y	HSA Binding (%) <sup>a</sup>	LD <sub>50</sub> (mmol/kg) <sup>b</sup>
Cholic	OH	OH	72	7.6
Lithocholic	H	H	98	0.66
Deoxycholic	OH	H	94.5	3.6
Chenodeoxycholic	H	OH	> 90	3.0 (2/5)

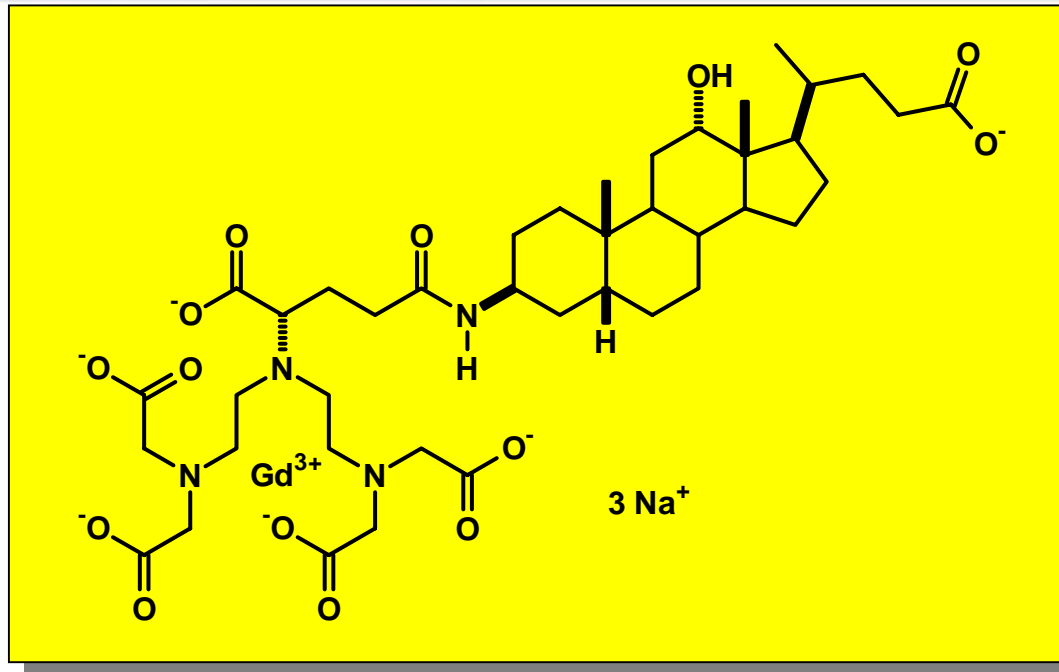


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M = Na or MegH

<sup>a</sup> by ultrafiltration; <sup>b</sup> in mice

# Deoxycholic acid derivative (B22956/1)



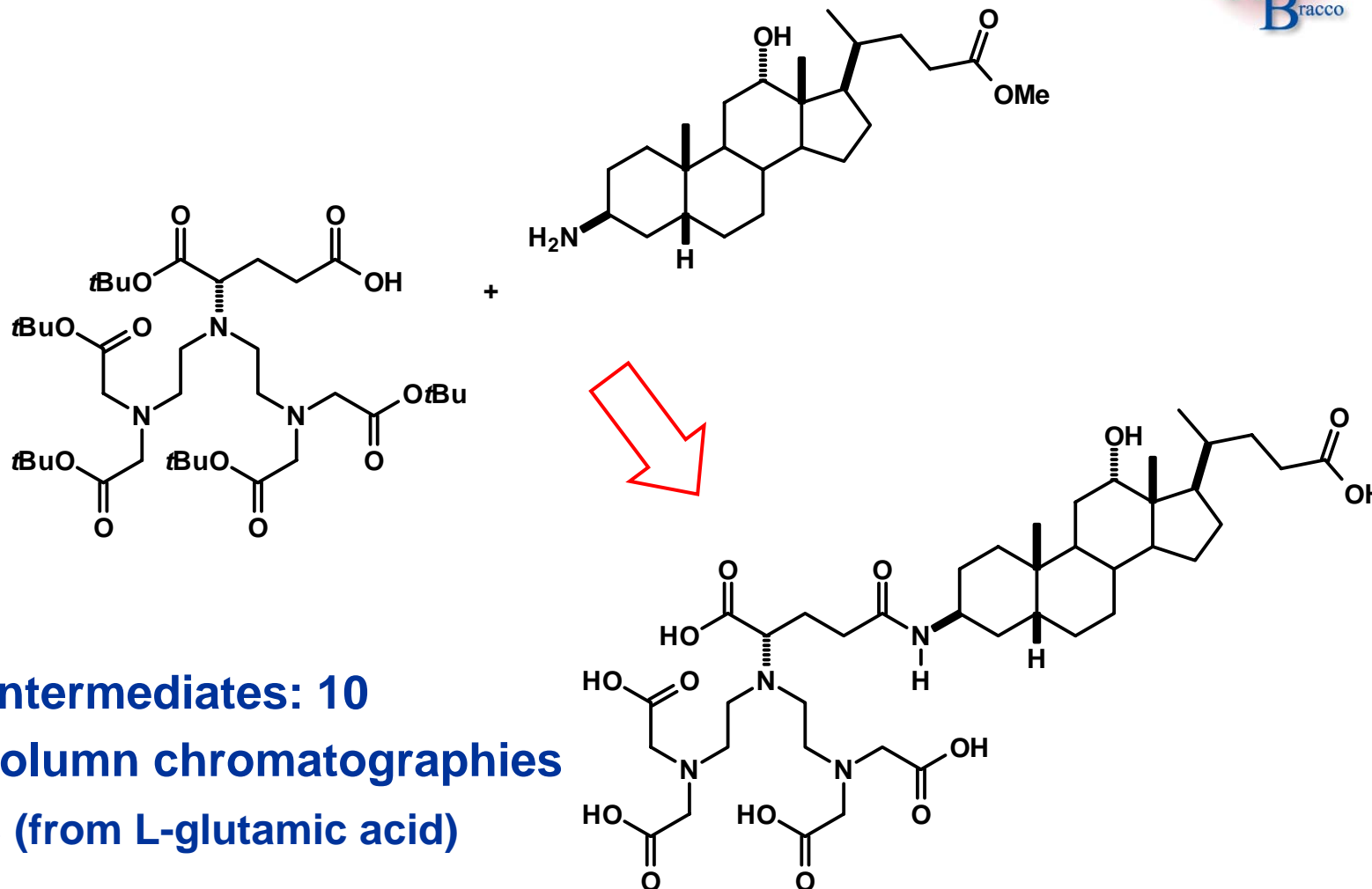
**Binding to HSA: 94.5 % ( $K_a$   $4.5 \cdot 10^4$ ,  $M^{-1}$ )**

**$[r_1$  in human serum:  $27 \text{ mM}^{-1} \text{ s}^{-1}$ ]**

**$LD_{50}$ :  $3.6 \text{ mmol/kg}$**



# Research Route to B22950



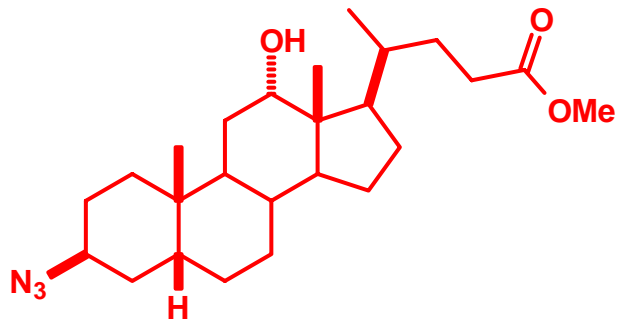
**Isolated intermediates: 10**  
**Several column chromatographies**  
**Yield: 6% (from L-glutamic acid)**



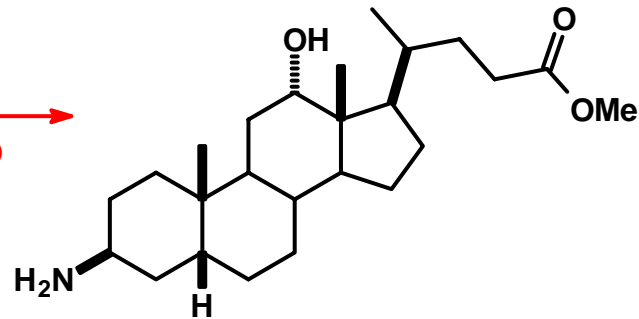
# R vs D routes – cholic moiety

**R**

DEAD, PPh<sub>3</sub>  
DPPA, THF

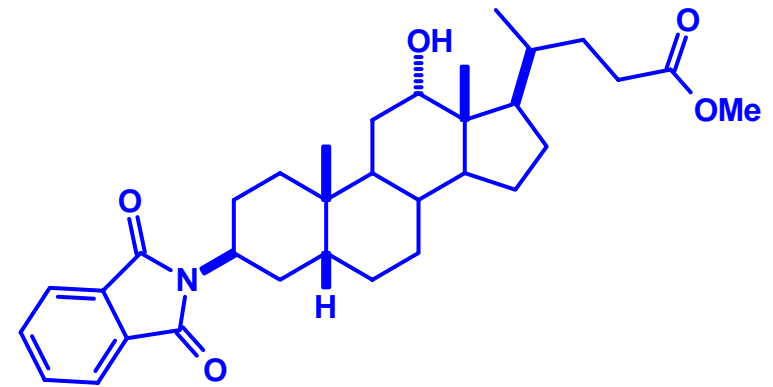


PPh<sub>3</sub>, H<sub>2</sub>O  
THF



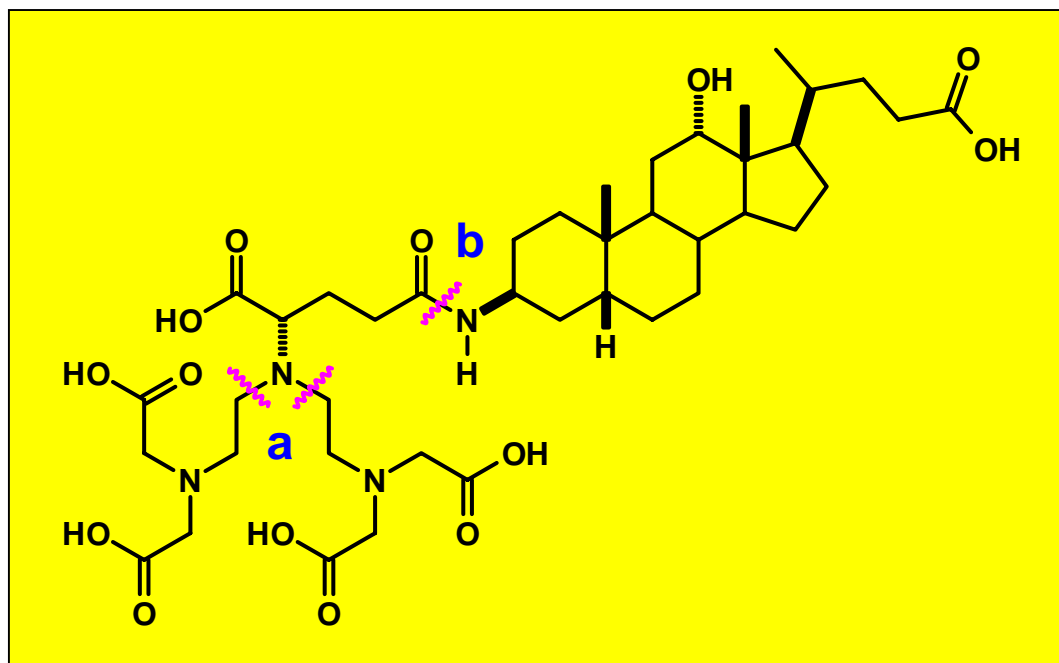
**D**

DEAD, PPh<sub>3</sub>  
Phthalimide, THF



1) NaBH<sub>4</sub>, DMA, pH 8 buffer  
2) HCl, MeOH

# Chelating moiety - disconnection approach



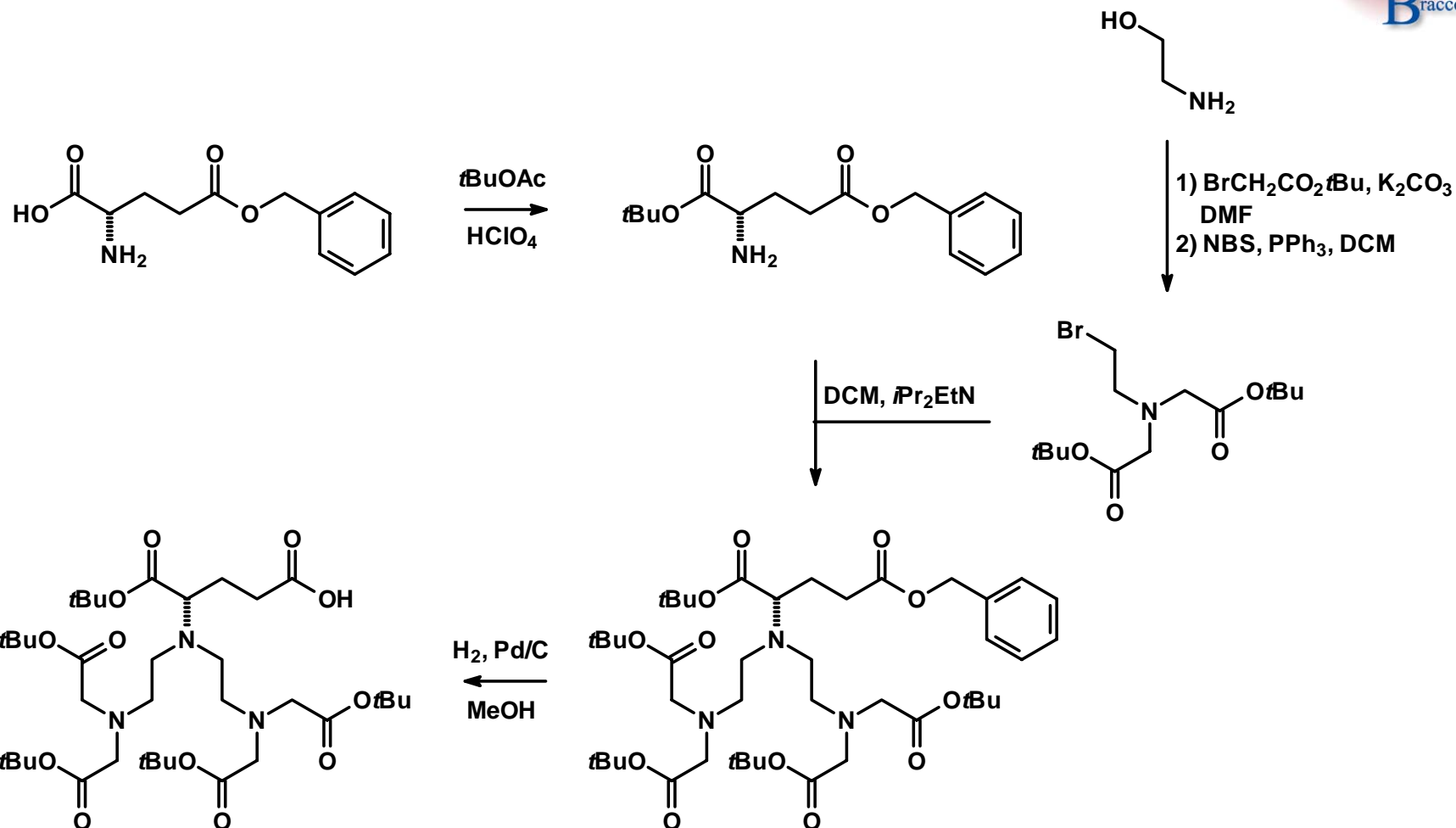
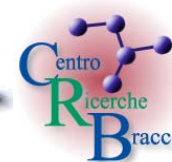
Research I) a II) b

Development I) b II) a

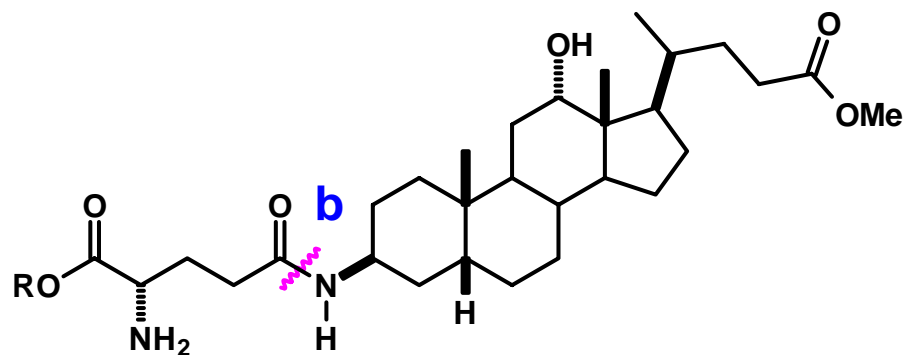


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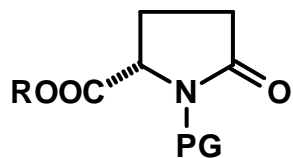
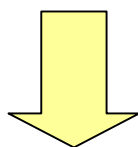
# Chelating moiety – research route



# Chelating moiety – development route 1

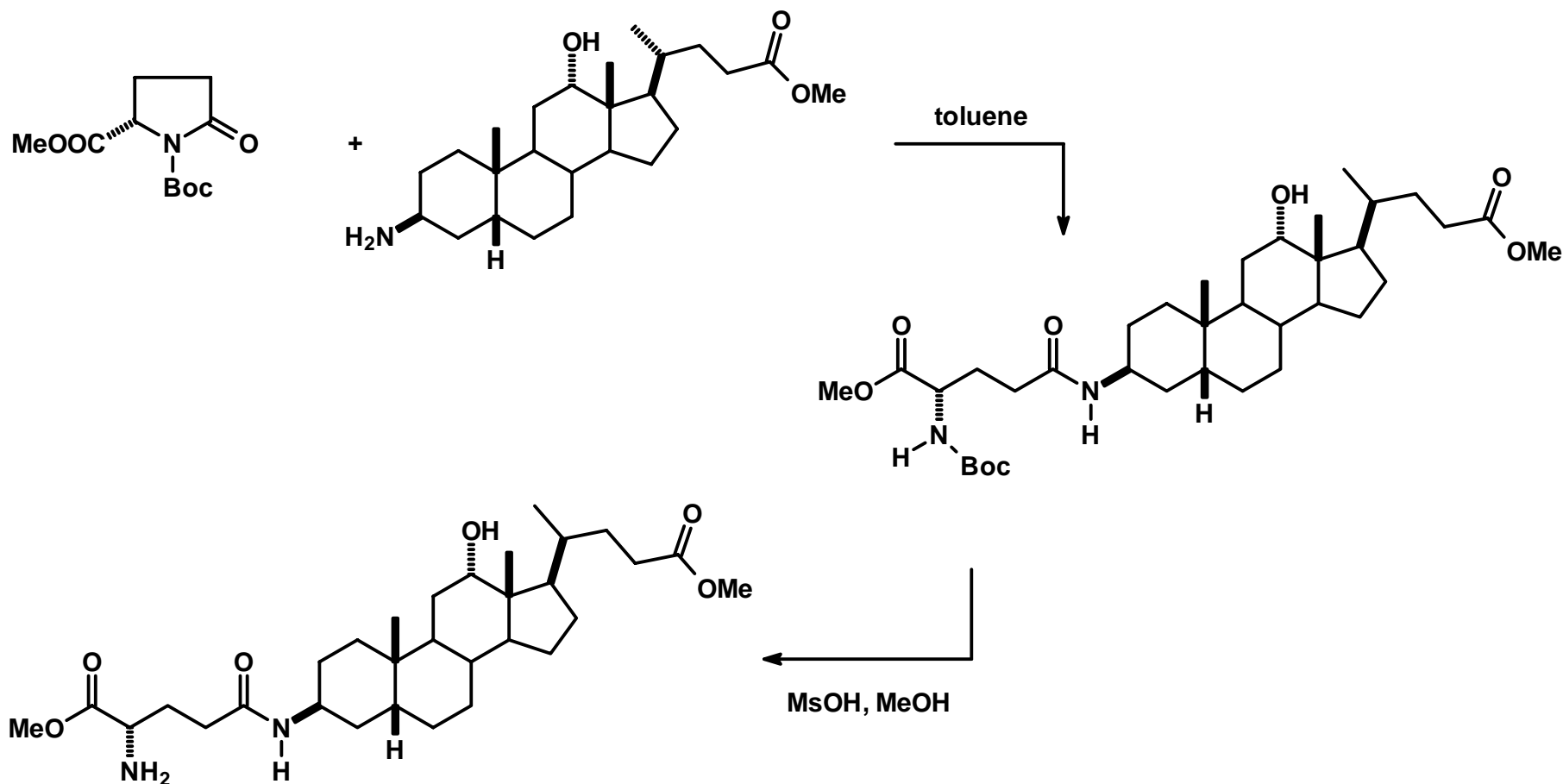
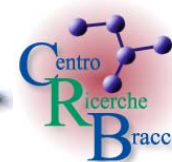


Looking for a convenient  
L-Glu derivative...



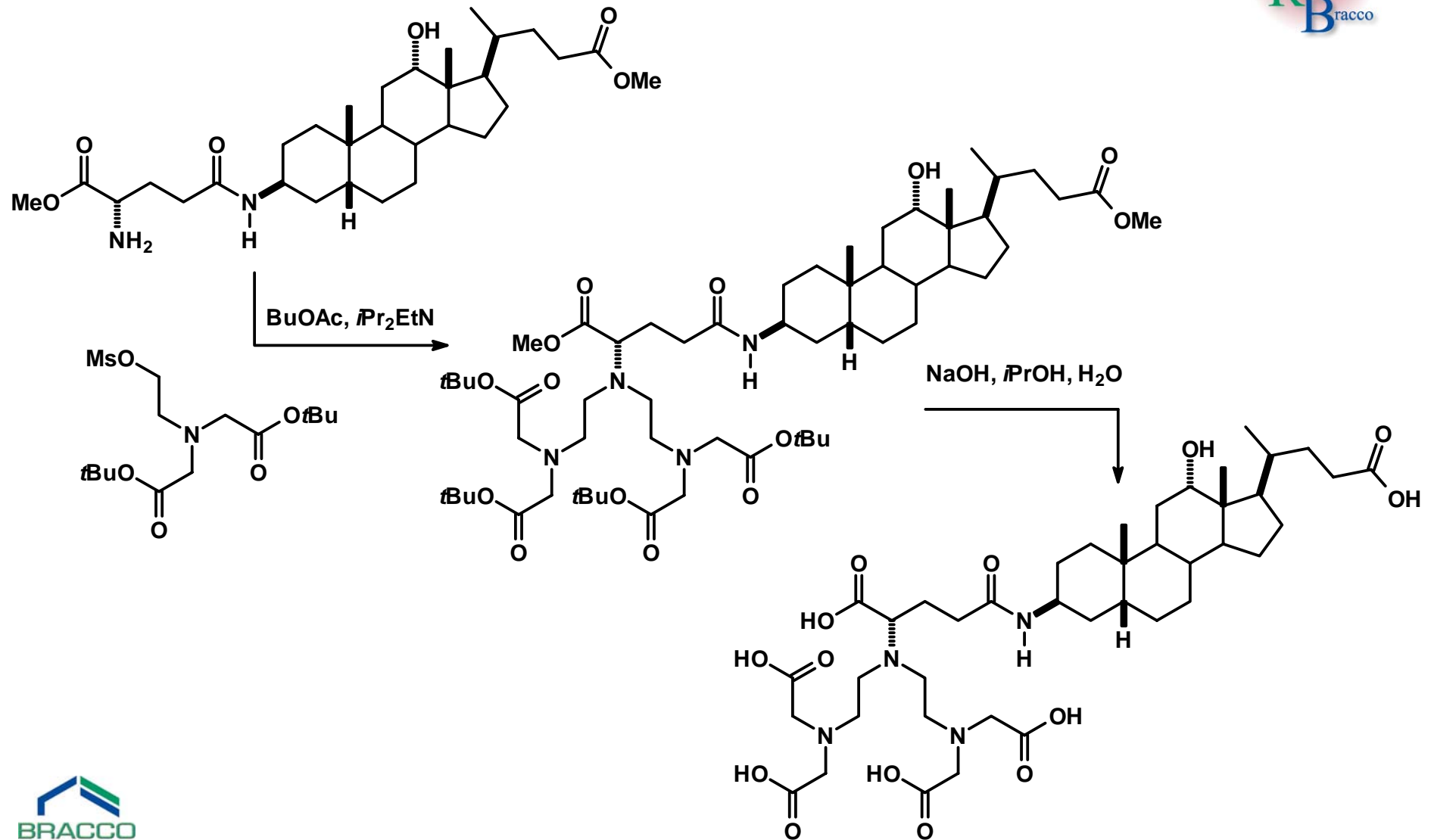
No coupling agent required

# Chelating moiety – development route 2



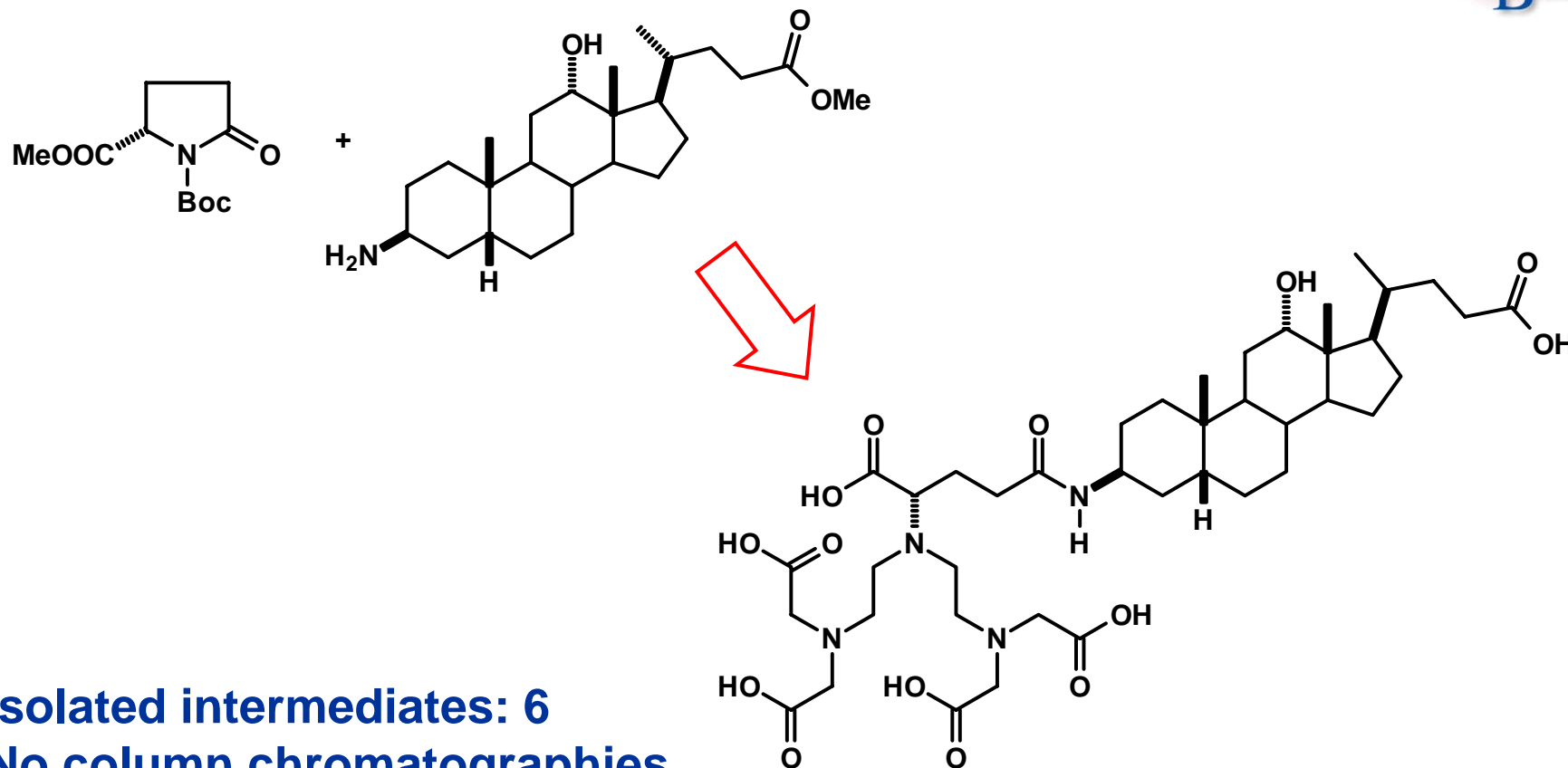
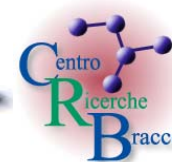
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# Chelating moiety – development route 3



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# Development route to B22950

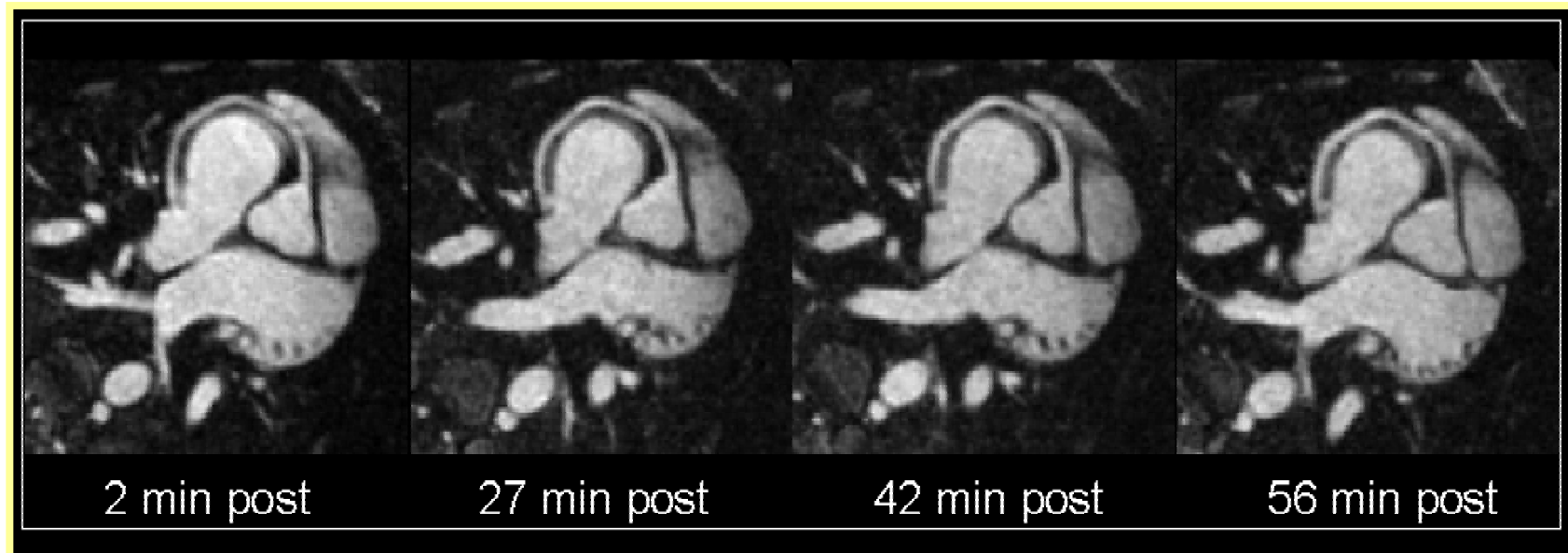


**Isolated intermediates: 6**  
**No column chromatographies**  
**Yield: 30% (from L-glutamic acid)**



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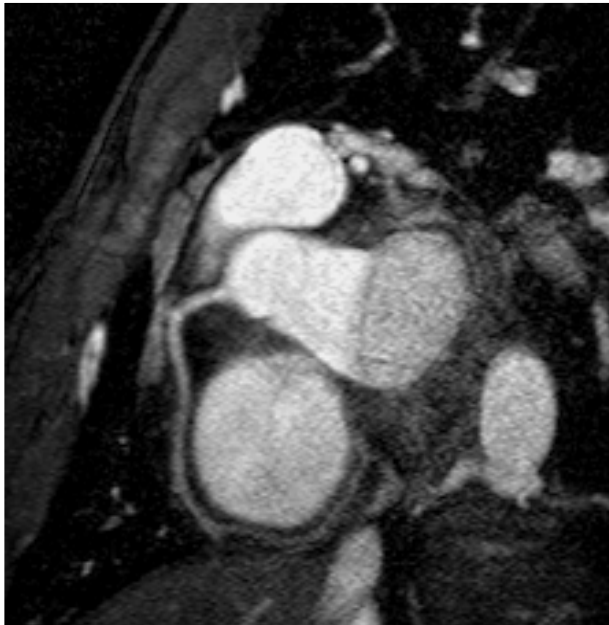
# B22956/1: preclinical studies



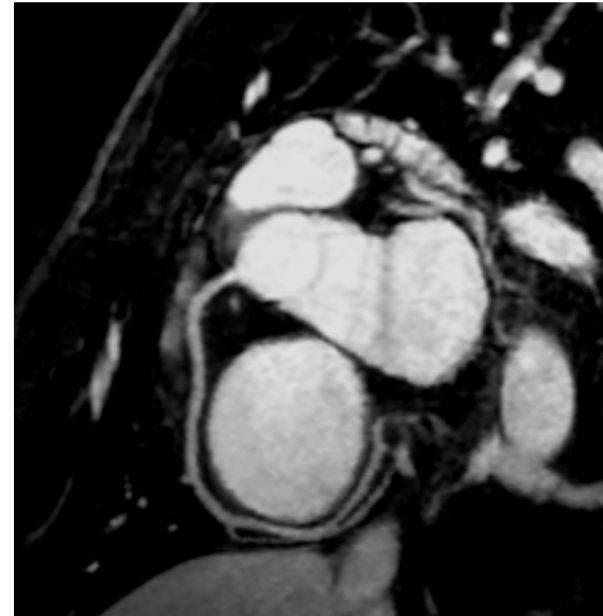
Multiplanar reconstruction from inversion-recovery 3D contrast enhanced MRCA after administration of B22956/1 (0.1 mmol/kg) to a micropig

# B22956/1: clinical studies

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T2-PREP precontrast



0.075 mmol/kg of B22956/1  
Inversion Recovery  
25 min postcontrast



MPR Images (Philips Gyroscan® 1.5T) 3D Navigator Gated and Corrected FFE

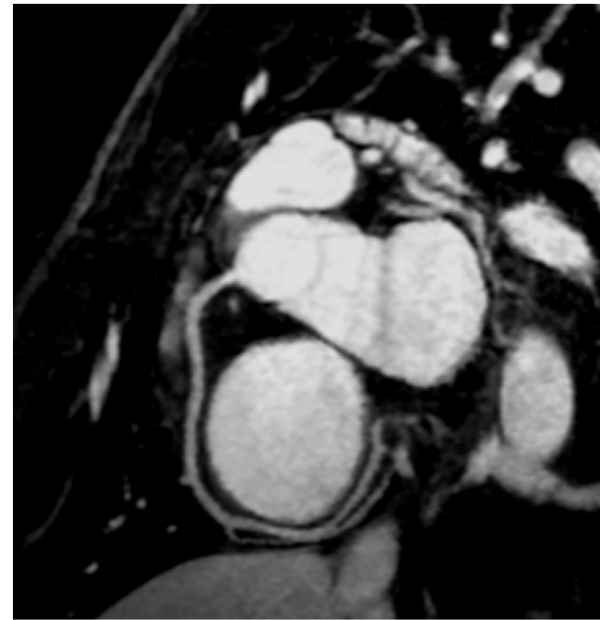
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# B22956/1: clinical studies



2

ProHance®



1

0.075 mmol/kg B22956/1  
Inversion Recovery  
25 min postcontrast



1. MPR Images (Philips Gyroscan® 1.5T) 3D Navigator Gated and Corrected FFE
2. 3D contrast-enhanced Breathhold IR-FLASH\*

\* Courtesy Debiao Li NWU



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