

## DEVELOPMENT OF A GENERIC AND SENSITIVE MONOCLONAL ANTIBODY FOR THE DETECTION OF TETRACYCLINES

P. Lowry, M.E. Benchikh, R.I. McConnell, A. Tohill and S.P. Fitzgerald

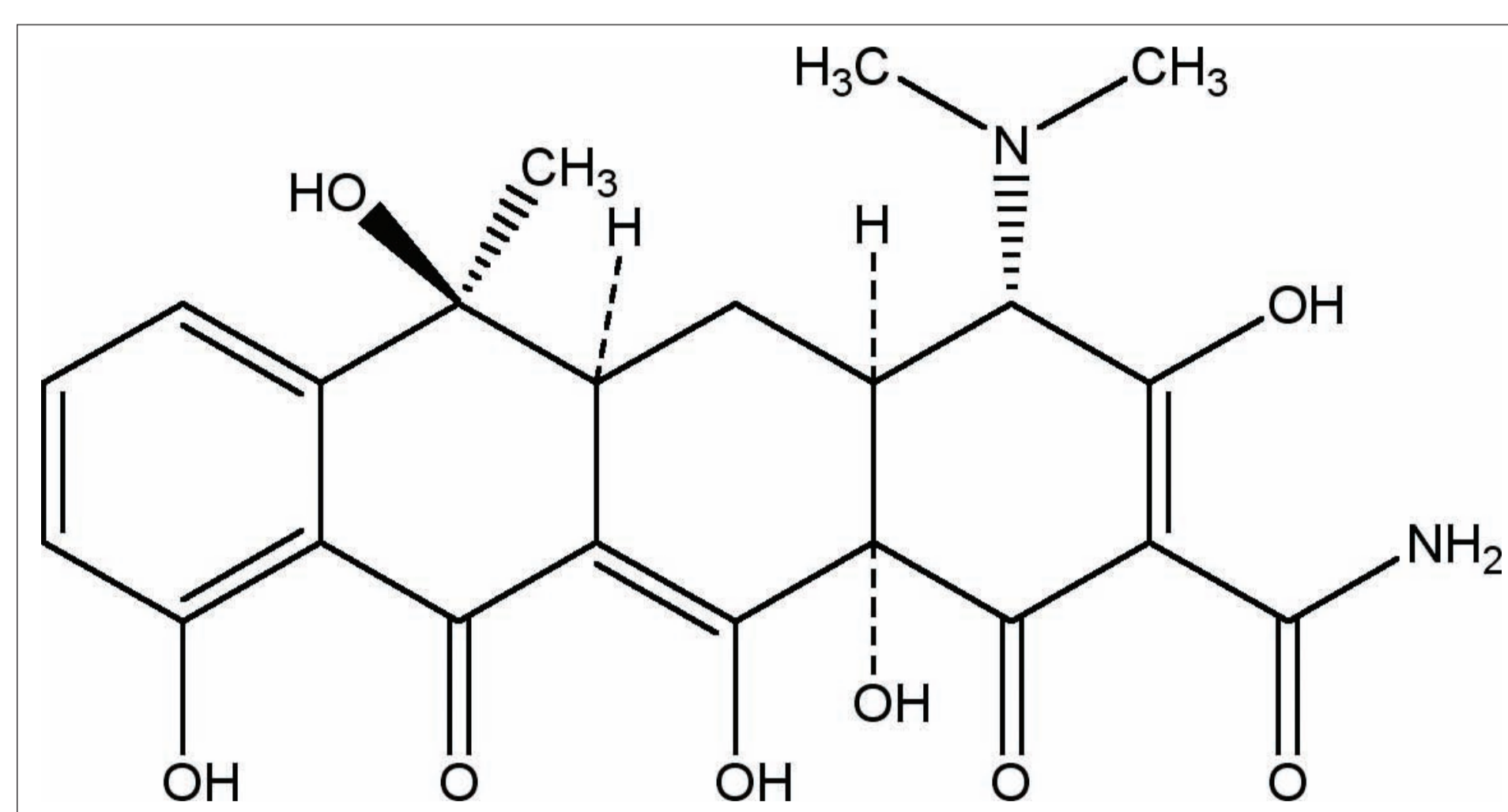
Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim BT29 4QY, United Kingdom

### Introduction

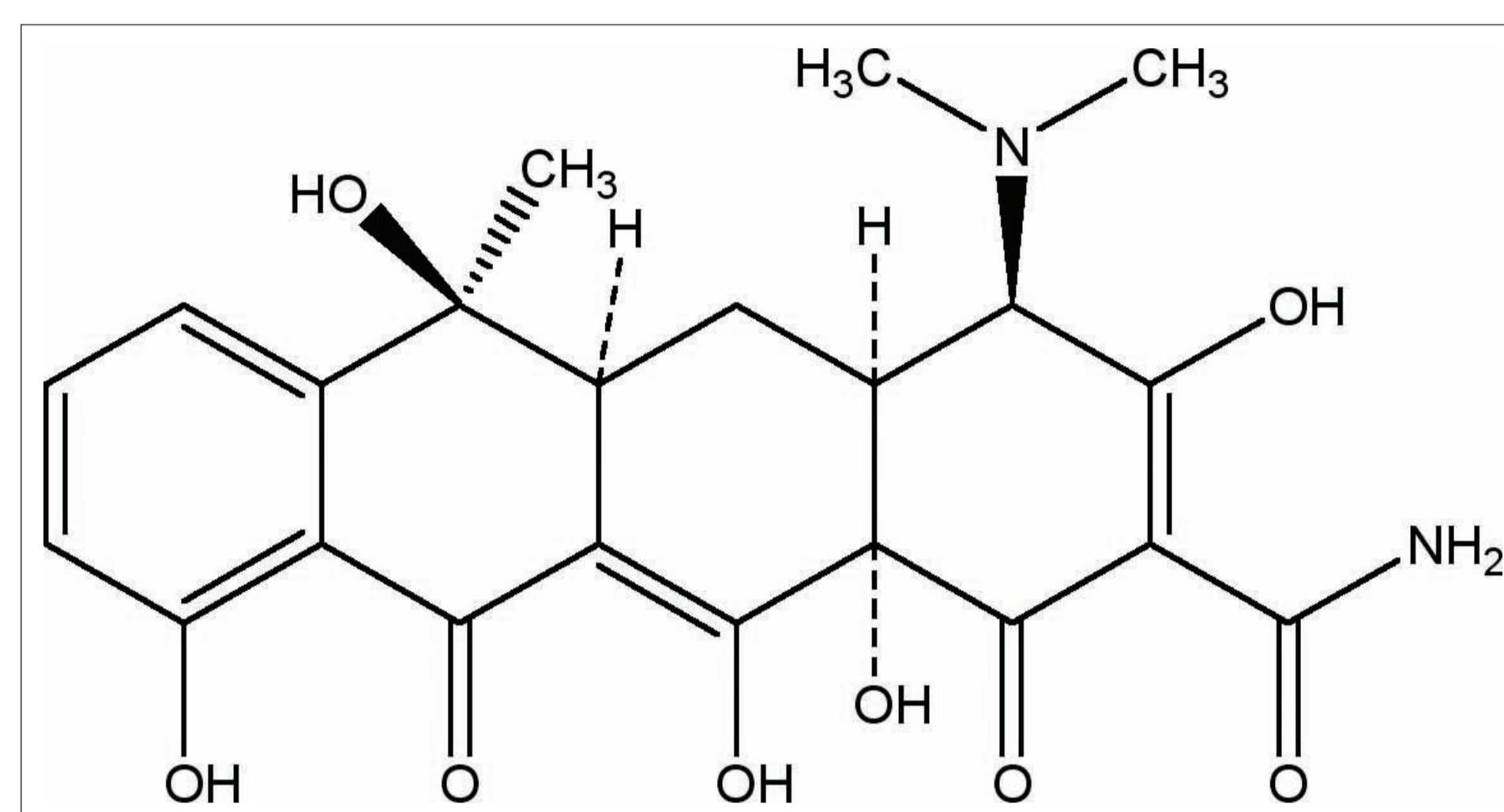
Tetracyclines are a group of broad-spectrum antibiotics, commonly used to treat disease in livestock. Due to potential contamination of the food supply, tetracycline antibiotic levels must be routinely monitored to ensure regulatory compliance.

We report the development of a monoclonal antibody presenting a broad specificity profile, which will be of value in developing more effective immunoassays for the detection of tetracyclines.

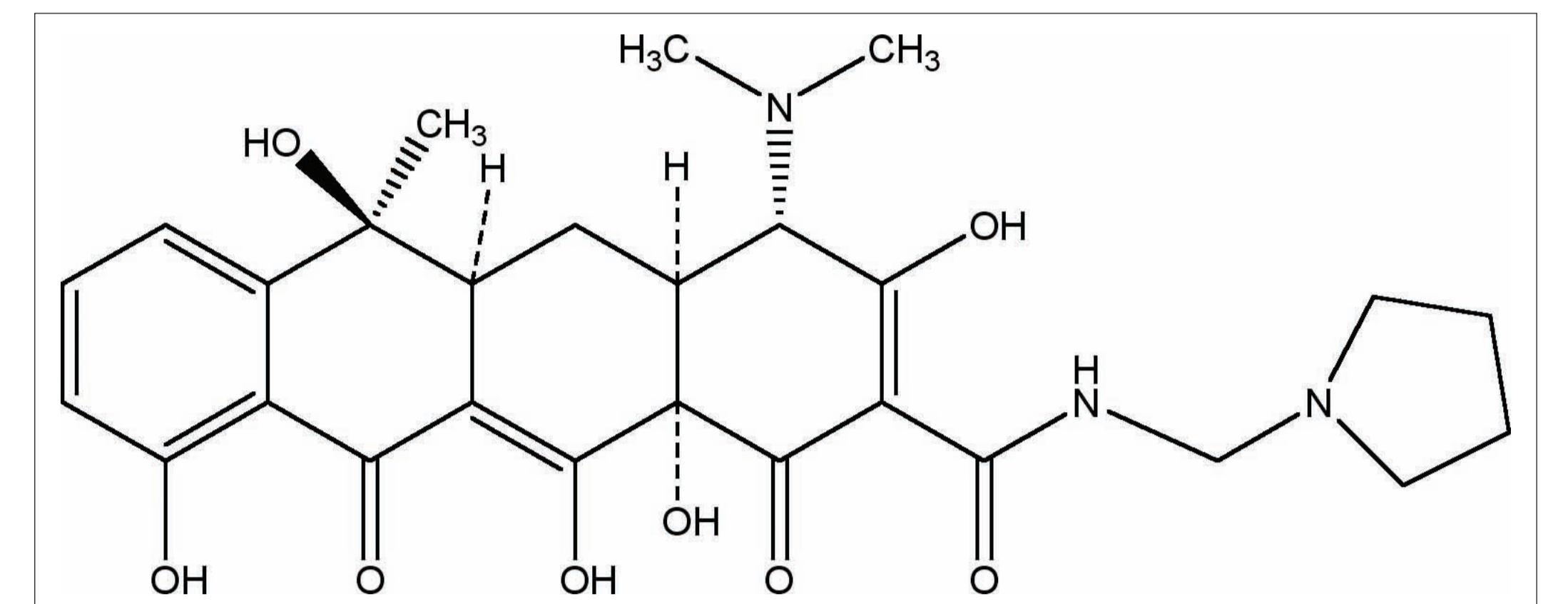
### Chemical Structures



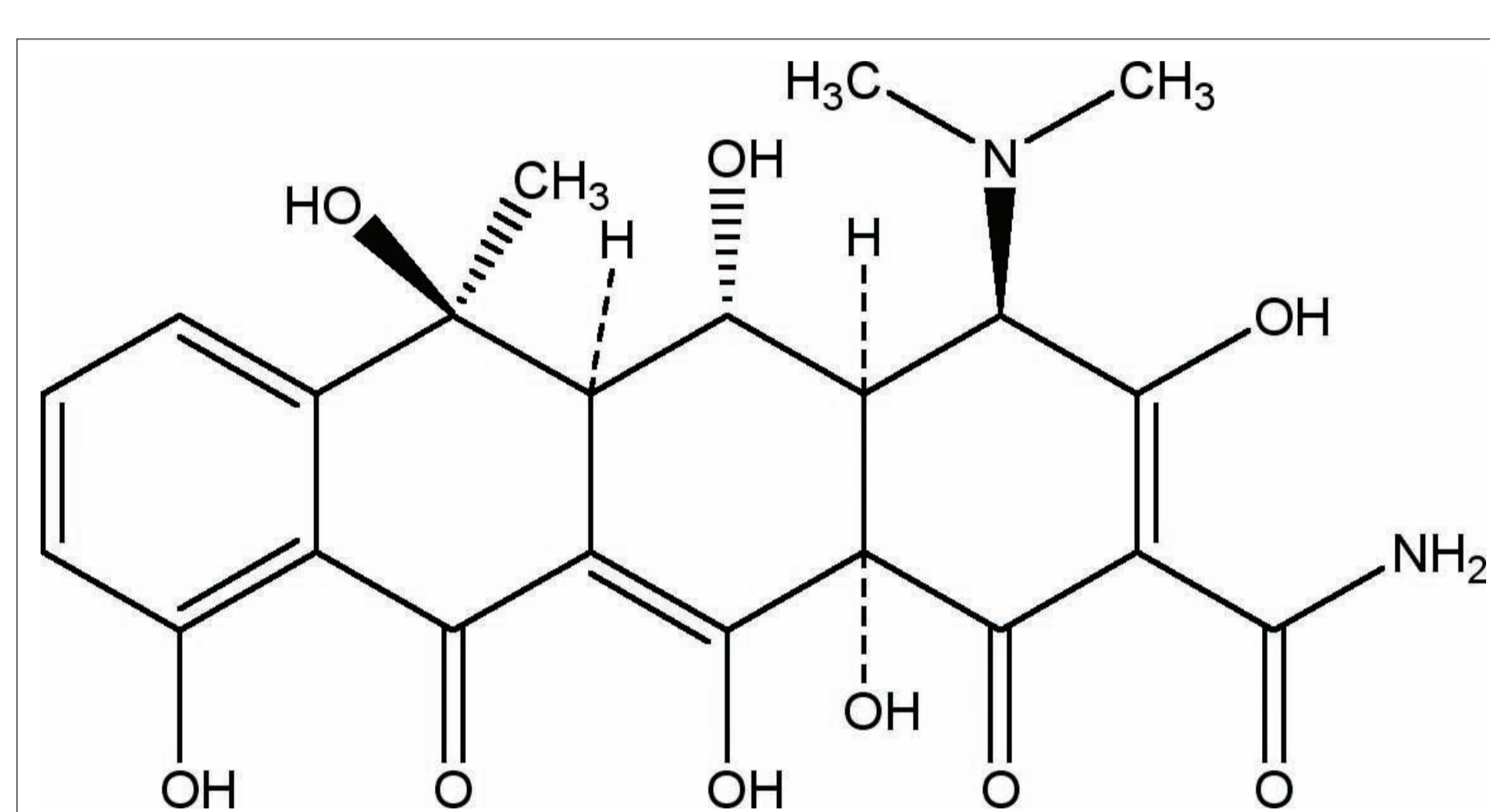
Tetracycline



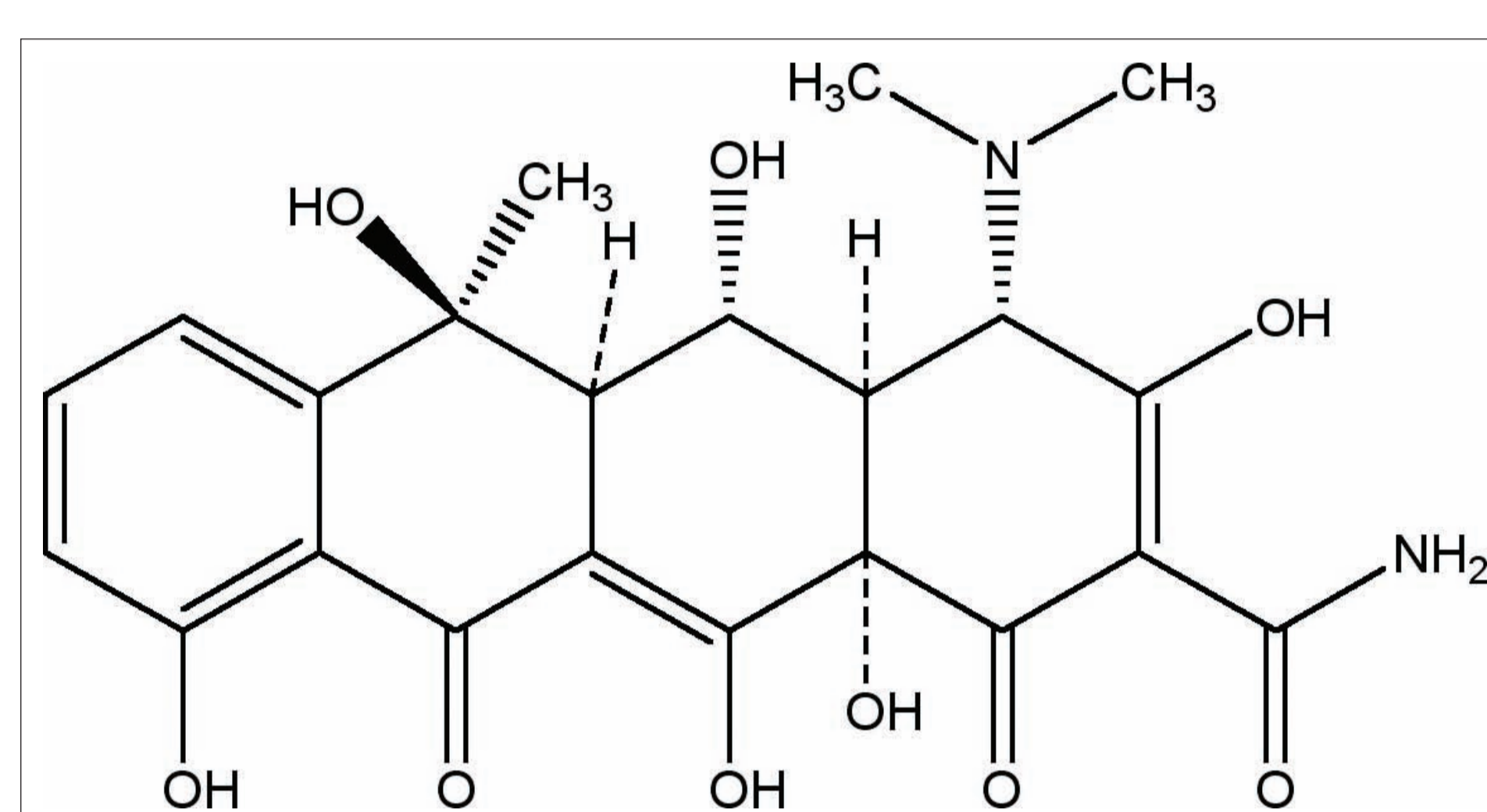
4-Epitetracycline



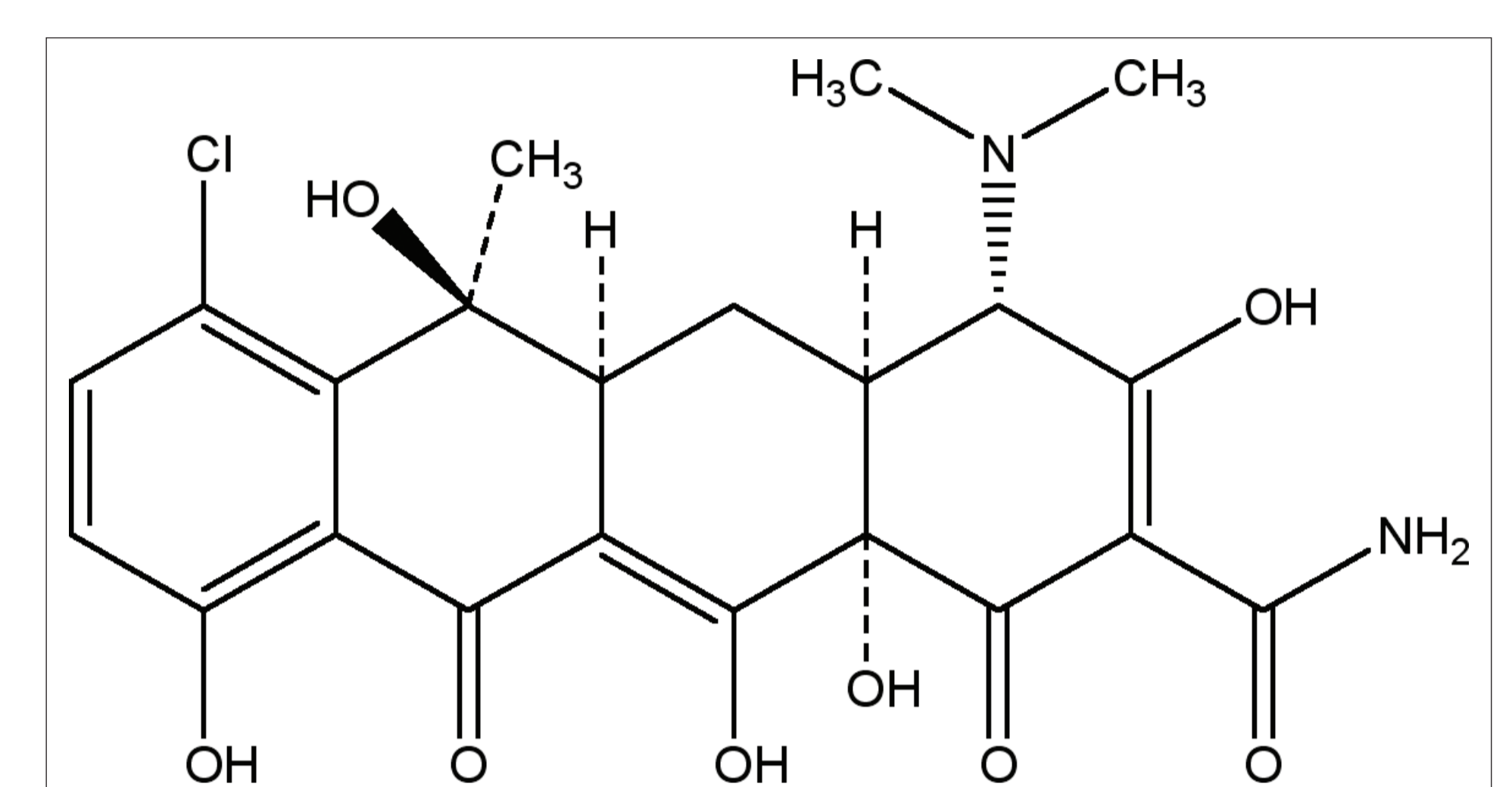
Rolitetracycline



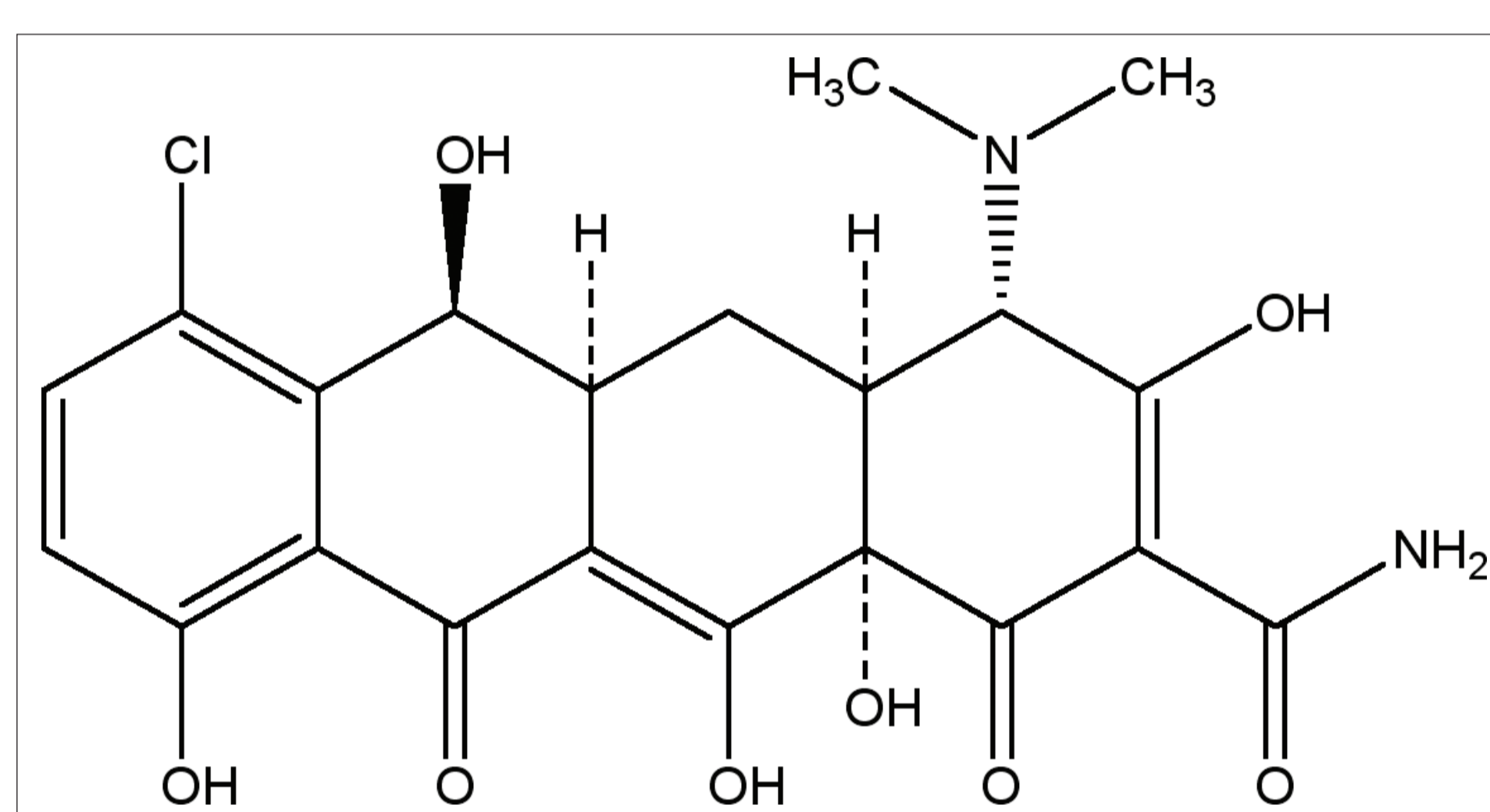
4-Epi-Oxytetracycline



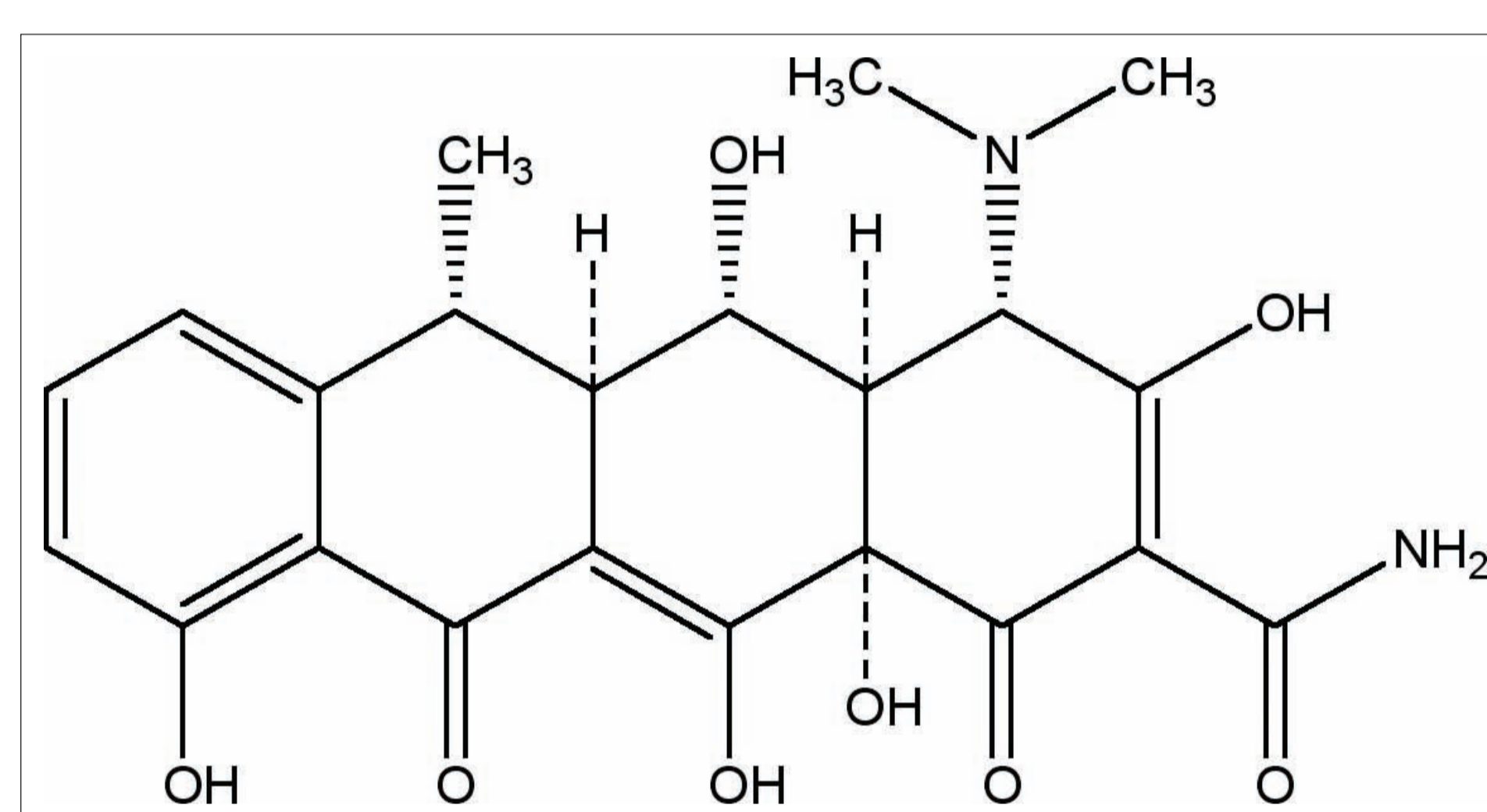
Oxytetracycline



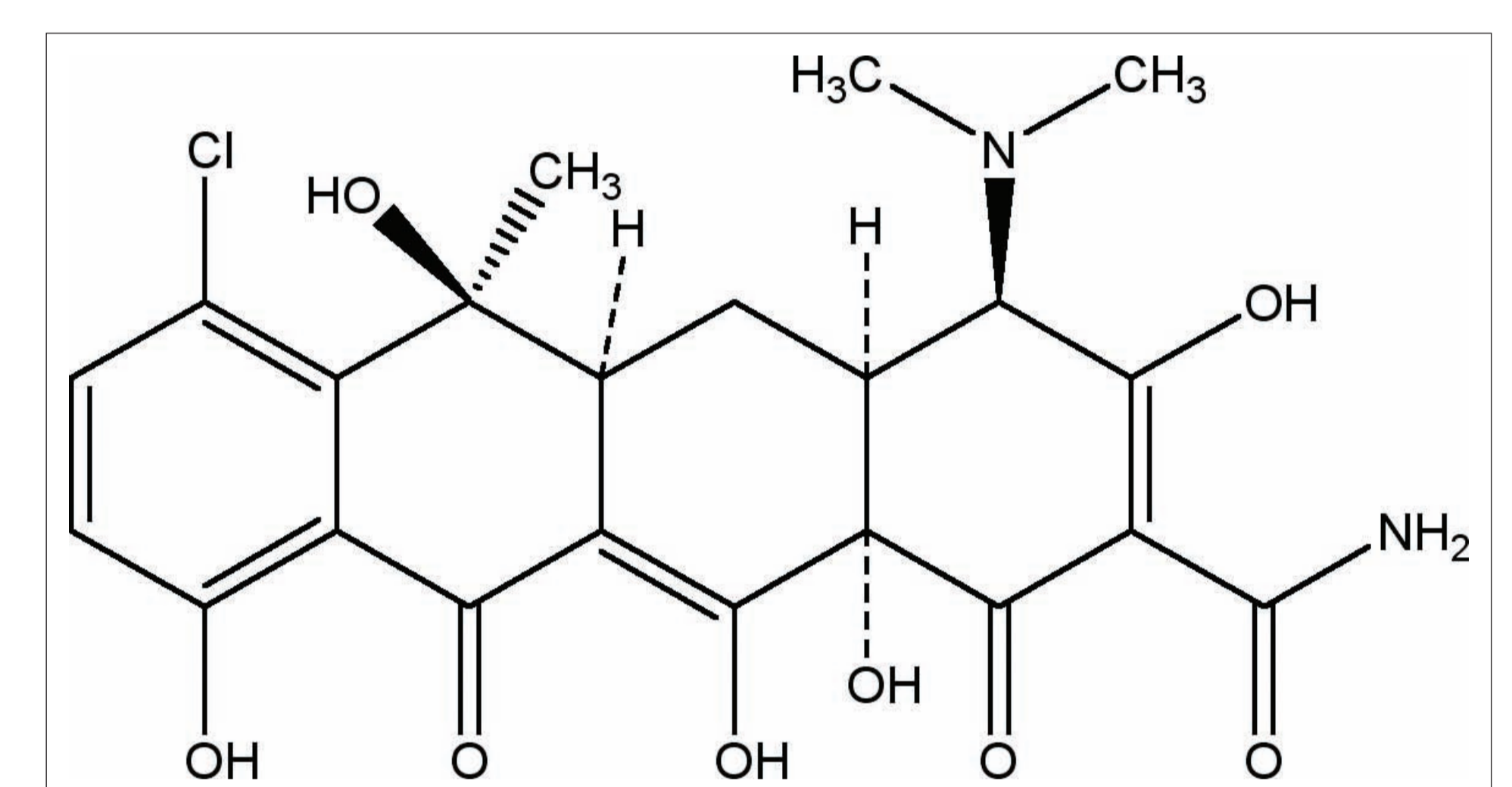
Chlortetracycline



Demeclocycline



Doxycycline



4-Epichlortetracycline

### Methodology

Sheep were immunized with tetracycline conjugated to bovine thyroglobulin (BTG) as a carrier. Lymphocytes were collected from pre-immunised animals and fused with heteromyeloma cells. The resulting hybridoma supernatants were screened for the presence of antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal cell lines. The antibodies were purified and evaluated by competitive chemiluminescent biochip array immunoassay on the Evidence Investigator analyser (EV3602, Randox Laboratories, Crumlin, Northern Ireland).

#### Assay evaluation parameters:

##### Sensitivity

The IC50 for each analyte was calculated by taking 50% of the signal from the zero calibrator and reading this value from the x-axis (concentration in ppb) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.

##### Specificity

Specificity: the specificity, expressed as %cross-reactivity (%CR) was calculated as follows:  
 $\%CR = [IC_{50}(\text{tetracycline}) / IC_{50}(\text{cross-reactant})] \times 100$

##### Precision

Intra-assay precision (n=20) was determined from the results of replicates at different concentration levels within the same run. Results were expressed as %CV.

### Results

Results corresponding to the initial antibody evaluation are presented.

#### Sensitivity

##### Monoclonal Antibody for Tetracyclines

Analyte	Calibration Range (ppb)	IC50 (ppb)
Tetracycline	0-2.5	0.28

08/29/13, 332,336, 338, 339, 341-343, 346, 348, 349, 371, 373, 375, 376, 378, 381, 385, 386, 390, 392, 394, 396, 401, 406-414, 416, 418, 419, 424, 426-428, 432, 434, 436, 439, 440, 446, 453, 456, 457, 460, 467, 471, 476/2/5

#### Specificity/Cross-reactivity (CR)

Analyte	%CR
Tetracycline	100
4-Epitetracycline	87
Rolitetracycline	67
Oxytetracycline	52
4-Epi-Oxytetracycline	52
Chlortetracycline	51
Demeclocycline	41
Doxycycline	23
4-Epichlortetracycline	20
Methacycline	11

08/30/17, 337, 382, 387/2/5

#### Intra-assay Precision

##### Intra-assay Precision (n=20)

Tetracyclines	%CV
Level 1	6.9
Level 2	7.6
Level 3	5.7

08/24, 332/2/5

### Conclusion

- Data indicate that the developed monoclonal antibody presents a broad specificity profile for tetracyclines such as tetracycline, 4-epitetracycline, rolitetracycline, demeclocycline, oxytetracycline, 4-epi-oxytetracycline, chlortetracycline, doxycycline, 4-epichlortetracycline with %cross-reactivities ranging from 100% to 20%

- The sensitivity value expressed as IC50 was 0.28 ppb.

- The monoclonal antibody developed can be used in the development of effective immunoassays for the detection of these compounds in test samples.